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SECTION MEETINGS

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11337

Movements of Human Diaphragm During Cardiac Cycle in
Respiratory Pause.

ANCEL KEYS.

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Minneapolis, Minn.*

Diaphragmatic "tug," accompanying ventricular systole in man, has been recognized for many years (*cf.*, *e.g.*, Mackenzie,¹). It has not been analyzed but it has been stated that it "can produce but little movement upward because of the inertia of the heavy abdominal organs." (Hamilton.²)

By means of the multiple slit roentgenkymograph of Stumpf³ the component of motion in any given plane of moving boundaries between regions of differing radiographic density can be accurately determined for intervals of time down to about 0.03 second. We

¹ Mackenzie, J., *Diseases of the Heart*, 1913, 2nd ed., London.

² Hamilton, W. F., *Am. J. Physiol.*, 1930, **91**, 712.

³ Stumpf, P., *Fortsch. Röntgenstr.*, 1928, **36**, 3.

2 MOVEMENTS OF DIAPHRAGM DURING CARDIAC CYCLE

have used this procedure for measuring the size change and stroke output of the heart during a single cardiac cycle (Keys and Friedell⁴).

Stumpf⁵ noted a cephalad movement of the diaphragm during systole and also stated that this movement may be reversed in the more lateral region of the diaphragm. We have studied a large number of roentgenkymograms (R.K.G.s) to get quantitative measures of these movements as recorded in posterior-anterior, lateral, and oblique views during respiratory pause, in all of which we have made both vertical and horizontal R.K.G.s at a film to target distance of 66 inches. Mathematical analysis of the measurements from tracings on these R.K.G.s was made by regarding the diaphragm as a frustrum of a dome of elliptical section. Rather than assume exact conformity to a simple geometrical form, the displacements recorded were integrated over a large number of steps.

Fig. 1 reproduces the tracing of the horizontal component of motion of both heart and diaphragm in the p.a. view in a typical case (normal young man 5 minutes after moderate exercise). Here, as is generally

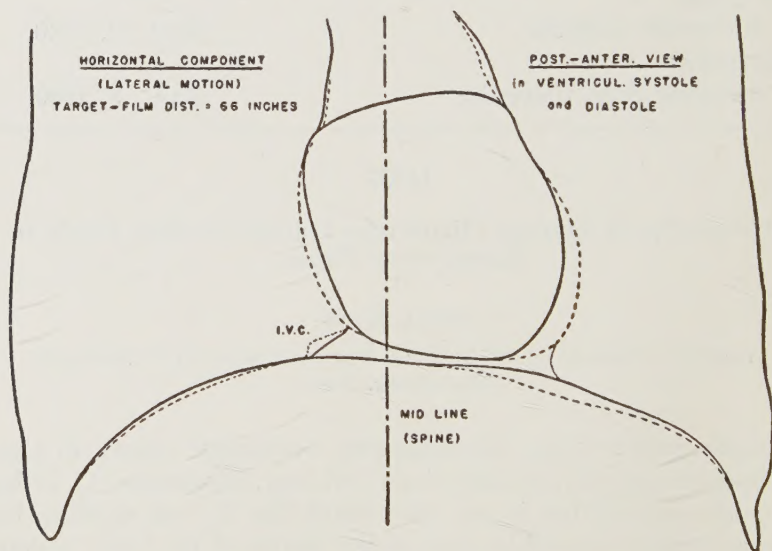


FIG. 1.

Horizontal component of motion of the heart and diaphragm, posterior-anterior view. Exact tracing of position of boundaries at full left ventricular diastole and the next succeeding systole during respiratory pause (moderate inspiration). Normal young man.

⁴ Keys, A., and Friedell, H. L., *PROC. SOC. EXP. BIOL. AND MED.*, 1938, **40**, 267; *Am. J. Physiol.*, 1939, **126**, 741.

⁵ Stumpf, P., Weber, H. H., and Weltz, G. A., *Röntgenkymographische Bewegungslehre innerer Organe*, 1936, G. Thieme, Leipzig.

the case, both right and left diaphragms moved laterally in systole and this motion is greatest (maximum 15.5 mm) on the left side near the apex of the heart. The vertical component of motion in the p.a. view in this case showed cephalad motion of the left and central diaphragm, greatest near the apex (maximum 4.5 mm), but with apparent paradoxical motion of most of the right diaphragm (maximum 3 mm). This again is generally the case, as is the appearance of paradoxical motion in the posterior portion of the central and left diaphragm when studied in the lateral R.K.G.

The total net motion of the diaphragm is always such as to reduce the volume of the thorax during systole. Since the 4 films necessary cannot be made simultaneously, exact values cannot be given but in the young man whose R.K.G. tracing is shown here (Fig. 1), the total net volume change of the thorax computed from the p.a. and lateral R.K.G.s was about 50 cc, of which approximately 80% was the resultant of lateral (horizontal) motion and 20% was pure vertical elevation of the diaphragm. The small value for the net vertical component results from the paradoxical vertical motion of the diaphragm. In this illustrative case the mean stroke output (left ventricle) was about 70 cc from our method of calculation (Keys and Friedell³). The volume reduction of the thorax during the cardiac cycle always corresponds to at least a large fraction of the stroke output. These observations on the diaphragm lend further support to our contention that the total heart size in man is markedly reduced in systole and that this reduction is closely related to the stroke output.

We have also studied 8 cases of pneumoperitoneum by these methods. In these cases the viscera are removed from the peritoneum by 5 to 15 cm and not only the free diaphragm but also the whole of the caudal portion of the heart are made sharply visible. The results are in full agreement with our observations on normal individuals where the heart and diaphragm are less fully visualized.

It should be noted that these diaphragmatic and cardiac movements in man are not necessarily identical with those in animals (Hamilton and Rompf⁶) which do not maintain the erect posture and which possess a different architecture about the heart (absence of rigid mediastinum, presence of a frenulum attachment to the apex of the heart, etc.). In man, alteration of posture or of the respiratory phase changes the form of the movement. For example, the movements of the diaphragm are more difficult to visualize in expiration and they tend to disappear in the Valsalva experiment. In all cases the total thoracic volume tends to diminish in systole by an amount

⁶ Hamilton, W. F., and Rompf, J. H., *Am. J. Physiol.*, 1932, **102**, 559.

which appears to correspond to a large fraction of the systolic discharge. During respiration these passive movements are obscured by the active movements of the diaphragm but if the stroke volume is large and respiration is very quiet traces may still be visible in the R.K.G.

Summary. In man during respiratory pause there are changes in the position of the diaphragm during the cardiac cycle so that in ventricular systole the total thoracic volume is smaller than in diastole. Calculation of the net thoracic changes involved integration of measurements of both vertical and horizontal components of motion in posterior-anterior and lateral projections. The reduction in thoracic volume amounts to a large and apparently rather constant fraction of the cardiac stroke output.

11338

**Production of Bradycardia in Normal Man by Neosynephrin*
(1- α -hydroxy- β -methylamino-3-hydroxy-ethylbenzene
hydrochloride).**

ANCEL KEYS AND ANTONIO VIOLANTE.

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Minneapolis, Minn.*

It is well known that heart rate and blood pressure do not rise in equivalent degree after administration of different sympathomimetic drugs. Ethylnor-suprarenin (3-4 dihydroxy-phenyl-1-amino-2-butanol-1) produces a rise in pulse rate and a fall in blood pressure (Cameron, *et al.*¹). Neosynephrin (3-hydroxyphenyl-1-methylamino-2-ethanol-1) increases the blood pressure with a relative fall in the pulse rate (Johnson²).

The production of relative bradycardia by sympathomimetic drugs has generally been ascribed to reflexes produced by the elevated blood pressure arising in the aortic arch and the carotid sinus. Such an effect can be demonstrated in man when small doses of epinephrine are used; the heart rate and blood pressure rise together but after some minutes the rate may fall while the blood pressure is still

* This work has been supported by a fellowship grant to the University of Minnesota by Frederick Stearns and Company.

¹ Cameron, W. M., Crismon, J. M., Whitsell, L. J., and Tainter, M. L., *J. Pharm.*, 1937, **62**, 318.

² Johnson, C. A., *Surgery, Gyn., Obst.*, 1936, **63**, 35.

above normal. When continuous electrocardiographic records are made before, during and after injection of a small dose of epinephrine in man, we have found an immediate but very transient slowing of the heart (Fuchs³). Large doses of some of these drugs may produce ventricular bradycardia as a result of block, cryptosystole and general cardiac damage.

We have studied the cardiac and vasomotor responses in normal man to epinephrine, neosynephrin hydrochloride and synephrin tartrate (*d*- α -hydroxy- β -methylamino-4 hydroxy-ethylbenzene tartrate). All studies were made in basal rest on 14 trained subjects, who received subcutaneous injections at intervals of several days. Each subject was studied at various dosages of all 3 drugs.

Synephrine tartrate produced no effects on pulse, blood pressure or the electrocardiogram in doses up to 60 mg. Epinephrine produced the classical results as well as the very transient slowing and occasionally the late reflex slowing mentioned above.

Neosynephrin consistently produced an immediate marked bradycardia which persisted from 30 to 90 minutes. Relative tachycardia never appeared except occasionally in very slight degree as the last effects of the drug were wearing off. The results were not changed

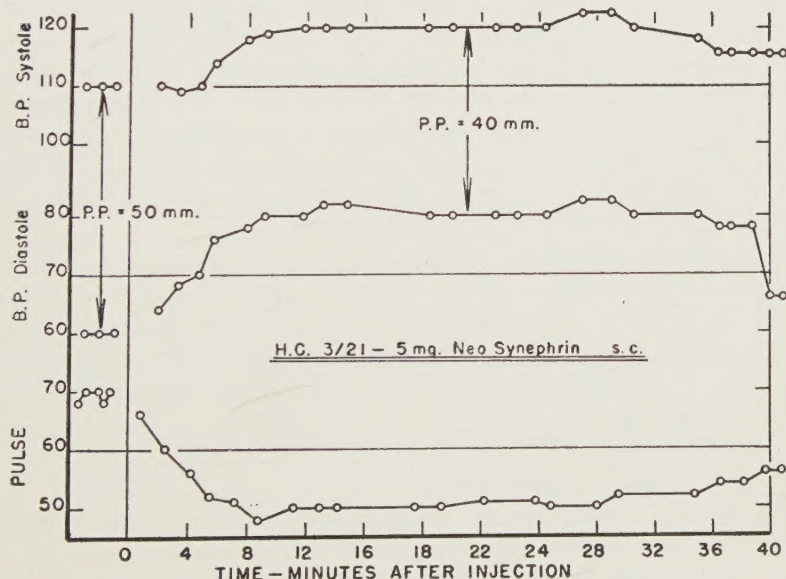


FIG. 1.

Typical course of blood pressure and pulse following subcutaneous injection of 5 mg of neosynephrin in a normal young man in the basal state.

³ Fuchs, R. T., *J. Pharm.*, 1938, **63**, 143.

when the subject was kept seated instead of prone. The threshold subcutaneous dose was from 1 to 2.5 mg in young adults from 110 to 180 lb in weight.

Fig. 1 shows the typical changes resulting from a rather small dose of neosynephrin. The pulse immediately starts to decline, reaching a constant low level at 7 to 10 minutes after injection. At the same time the diastolic blood pressure rises but the systolic pressure does not rise until the diastolic pressure and pulse changes are well established. With these rather small doses the systolic pressure rise is small and the pulse pressure is generally diminished. With a 10 mg injection (as is frequently used for the clinical dose) the initial time course is the same but the systolic pressure continues to rise so that the pulse pressure is eventually increased above normal. With the 10 mg dose the pulse rate may fall to 30 beats per minute and be maintained at 35 to 45 for as long as 80 minutes.

The electrocardiographic records are interesting. The rhythm is perfectly normal with no change in the PR interval though the RT interval (duration of systole) may be very slightly prolonged. There is no slurring of QRS in any of the leads at any time. Very rarely there may be inversion of P, especially in lead 3. Aside from the extreme bradycardia, the most notable change is a marked elevation of the T wave in all leads and a diminution of the P wave. In several cases the P wave practically disappeared and the E.C.G. would indicate A.V. nodal rhythm. Neither A.V. nor bundle branch block appeared. In a single case when a 10 mg dose was given to a small athletic woman there was a short period when the E.C.G. record could be interpreted as showing retrograde conduction or complete A.V. dissociation at equal rhythm.

The general appearance is that neosynephrin produces a primary bradycardia by inhibition of the sino-auricular node and this is relatively independent of blood pressure reflexes over the vagus nerve. Neosynephrin does not appear to accentuate the carotid sinus reflex; Nathanson⁴ reported it is effective in preventing syncope resulting from pressure on the carotid sinus. No distress or subjective excitement was reported by the subjects, several of whom were trained observers, in these extreme bradycardias.

Summary. Neosynephrin injected subcutaneously into normal, trained subjects in the basal state produces an immediate bradycardia and rise in diastolic pressure; systolic pressure rises later. The threshold is from 1 to 2.5 mg and pulse rates from 30 to 45, per-

⁴ Nathanson, M. H., *Arch. Internal Med.*, 1936, **38**, 683.

sisting for as long as 80 minutes, are produced by 5 to 10 mg. The E.C.G. remains normal with no change in A.V. or ventricular conduction time but there is a fall in the potential of the P wave and a rise in the T wave.

11339

A Method of Separating Small Quantities of the Coproporphyrin Isomers 1 and 3.

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Quantitative separation of the naturally occurring coproporphyrin isomers (1 and 3) has hitherto been impossible. Crystallization has usually permitted identification of the porphyrin predominating in any given mixture, such as obtained for instance from urine and feces.¹⁻⁴ This, however, has required that relatively large amounts of porphyrin be available. The present investigation was undertaken with the purpose of finding a means by which mixtures consisting of as little as 5-10 γ of total coproporphyrin could be resolved quantitatively.

We have found that the methyl esters of coproporphyrins 1 and 3 are quantitatively adsorbed on Brockmann's Al_2O_3^* under the conditions noted in the following. The ester of coproporphyrin 3 may be eluted quantitatively with 35% acetone in water while that of copro-1 remains adsorbed, and is later removed by elution with pure acetone. The various steps in the procedure are as follows: (1) Esterification of the total, free porphyrin mixture in methyl alcohol saturated with HCl gas. (2) Dilution with equal volumes of distilled water, followed by neutralization of the HCl with a saturated aqueous solution of sodium acetate, which is added drop by drop with constant stirring until the solution no longer turns Congo paper blue. Ten percent NH_4OH is then added drop by drop until the mixture becomes pink to phenol red. (A few drops of an aqueous

¹ Watson, C. J., *J. Clin. Invest.*, 1935, **14**, 106.

² Watson, C. J., *J. Clin. Invest.*, 1936, **15**, 327.

³ Dobriner, K., *J. Biol. Chem.*, 1936, **113**, 1.

⁴ Watson, C. J., *J. Clin. Invest.*, 1937, **16**, 383.

*Merck and Company, Inc.

solution of the latter indicator having been added to the entire mixture.) (3) The faintly alkaline solution is at once run through the column of Brockmann's Al_2O_3 , designated "a" in the accompanying diagram (Fig. 1). The column is next washed with 15-20 cc of distilled water. (4) The copro-3 ester is then removed by repeated washing with 35% acetone, as long as any red fluorescence is visible at b (Fig. 1). The total copro-3 fraction is collected in the lower suction flask and removed, after which elution with pure acetone is carried out in the same way. Relatively large amounts of 35% acetone are necessary for the copro-3 fraction. (5) The amount of porphyrin in each of the final solutions is then measured fluori-

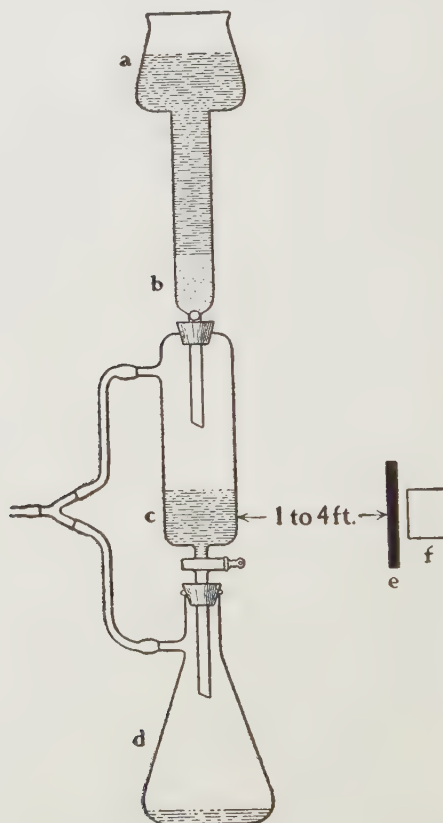


FIG. 1.

Apparatus for adsorption and elution. *a*. Fluid from which porphyrins are to be removed. *b*. Column of Brockmann's Al_2O_3 (a cotton wad is inserted in the neck of the tube just below the Al_2O_3). *c*. Fluid to be inspected for red fluorescence in UV light. *d*. Receiving suction flask. *e*. Corning red purple ultra filter No. 587. *f*. Carbon arc lamp.

metrically. In the present study a Zeiss stufenphotometer† has been used, and comparisons have been made with standard solutions of copro-3 ester in 35% acetone and copro-1 ester in pure acetone. The limit of error is within $\pm 3\%$. The intensity of fluorescence is about twice that of the free porphyrin in 1% HCl. The chief objection to the measurement of red fluorescence with the stufenphotometer⁵ is that the eye fatigues rather rapidly and more than a few readings cannot be taken at any one time. Other methods of measurement are being investigated.

A summary of data obtained in a number of recovery experiments carried out with the above described method is given in Table I.

It is not possible as yet to report data on the application of the above method to natural material. We have ascertained that considerable purification of the free coproporphyrin is necessary, preliminary to esterification and subsequent separation of the isomers. Investigation is now in progress to determine as simple a method of purification as possible, which will still be generally applicable.

The Al_2O_3 -acetone procedure is of much value in separating small amounts of copro-esters 1 and 3 for purposes of melting point determination and observation of crystal habitus. The data in Table II is evidence of the specificity of 35% acetone in eluting the copro-3. Extensive purification, consisting of repeated fractionation between ether and 1% HCl in the usual way¹ was carried out in each of these instances.

TABLE I.
Recovery of Copro-1 and 3 Esters from Various Mixtures by Al_2O_3 -Acetone Method.

No.	Amount Copro-1 used in γ	Amount Copro-1 recovered in γ	% recovery	Amount Copro-3 used in γ	Amount Copro-3 recovered in γ	% recovery	% Copro-1 in mixture
1	8.8	8.97	102	0.0	0.0	—	100
2	10.0	10.2	102	0.0	0.0	—	100
3	30.0	30.9	103	10.0	10.1	101	75
4	20.0	18.0	90	20.0	20.8	104	50
5	5.3	5.19	96	14.5	14.2	98	27
6	10.0	9.5	95	30.0	30.0	100	25
7	1.8	1.48	82	8.4	8.07	96	18
8	5.0	5.05	101	35.0	32.55	93	12.5
9	0.0	0.0	—	18.0	18.18	101	0.0

† The light source was a small, high pressure mercury arc lamp ("Mico" type) firmly attached to the front of the photometer. The light was filtered through a heat resisting red purple ultra filter, Corning No. 587.

⁵ Fikentscher, R., and Franke, K., *Klin. Wchnschr.*, 1934, 922.

TABLE II.
Crystallization of Coproporphyrin Esters After Separation by Means of Al_2O_3 -Acetone Method.

No.	Copro-1 ester			Copro-3 ester			Remarks
	Amt in γ (stufenphotometer)	M.P. in $^{\circ}\text{C}$	Crystal habitus	Amt in γ (stufenphotometer)	M.P.* in $^{\circ}\text{C}$	Crystal habitus	
1	40.3 (11%)	Not crystallized		349 (89%)	131	Prisms	Collected urines from 3 patients receiving sulfanilamide
2	265.0 (47%)	236-40	Fine curving needles	302 (53%)	150-60	Straight prisms and rosettes	Undulant fever; sulfanilamide; toxic reaction with jaundice
3	88.0 (16%)	Not crystallized		434 (84%)	152	Straight prisms and rosettes	Rheumatoid arthritis; gold therapy
4	175.0 (25%)	235	Rosettes of fine curving needles	534 (75%)	157-61	Straight prisms and rosettes	Hodgkin's disease under x-ray therapy
5	Relative amount only (43%)	242-4	Fine curving needles	Relative amount only (57%)	137-40	Straight prisms and rosettes	Rheumatic fever

*Copro-3 methyl ester exhibits dimorphism in melting point, i. e., 135°C , 144°C , $167-70^{\circ}\text{C}$.⁶

⁶ Fischer, H., and Orth, *Die Chemie des Pyrrols*, Bd. II, erste Hälfte. Akad. Verlagsgesellsch., Leipzig, 1937.

Polycythemia of Morbus Caeruleus (Cyanotic Type of Congenital Heart Disease.)

PHILLIP HALLOCK. (Introduced by C. J. Watson.)

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Minneapolis, Minn.*

The polycythemia of morbus caeruleus is similar in many respects to that of the primary form (polycythemia vera), yet from an etiological point of view it differs a great deal. In both forms the red cell counts and hemoglobin concentrations are increased and the hematocrit readings are high. Blood volume studies have revealed that the total blood volume and circulating red cell mass are considerably elevated in polycythemia vera while the plasma volume remains essentially unaltered.^{1, 2, 3} While it is repeatedly stated that the total blood volume and red cell mass are elevated in the polycythemia of morbus caeruleus, these assertions are based not on actual blood volume determinations but on inferences drawn first, from the fact that the red cell counts and hemoglobin concentrations are high, and second, from the assumption that morbus caeruleus is similar to other forms of secondary polycythemia which arise from oxygen deficiency. Extremely meager information is available concerning the status of total blood volume, circulating red cell volume, and plasma volume in morbus caeruleus. The first case of morbus caeruleus in which total blood volume was measured and in which an attempt was made to estimate the plasma volume was that reported by Parkes-Weber and Dorner.⁴ In this case of morbus caeruleus the total blood volume, as determined by Haldane and Smith's⁵ carbon monoxide method, was found to be increased. The plasma volume was determined indirectly and considered to be normal from the estimation of the concentration of the dry residue in the blood serum. One year previous to this, however, Bie and Maar⁶ had studied the concentration of plasma in 2 cases of morbus caeruleus by similarly estimating the concentration of the dry residue in the serum. They concluded that there was no significant change in plasma volume concentration

¹ Rowntree, L. G., and Brown, G. E., *The Volume of Blood and Plasma in Health and Disease*, W. B. Saunders Co., Philadelphia, 1929.

² Lampe, W., *Deutsche Med. Wchnschr.*, 1925, **51**, 2025.

³ Gibson, J. G., Harris, A. W., and Surgert, V. W., *J. Clin. Invest.*, 1938, 18.

⁴ Parkes-Weber, F., and Dorner, G., *Lancet*, 1911, **180**, 150.

⁵ Haldane, and Smith, L., *J. Physiol.*, 1900, **25**, 33.

⁶ Bie, W., and Maar, W., *Deutsches Arch. fur Klin. Med.*, 1910, **44**, 382.

and that the increased cell count was not, therefore, due to diminution in plasma volume. Blumenfeldt and Wolheim,⁷ using trypan red dye estimated the blood volume in a 20-year-old girl with cyanotic type of heart disease. The plasma volume was markedly diminished, namely, 690 cc (13.4 cc per kg). The total blood volume was 3,028 cc or 59.1 cc per kg. Meyer,⁸ in a study of the hemodynamics of the circulation in a 22-year-old male having Tetralogy of Fallot, estimated the plasma volume by means of the Congo red method and found it to be exceedingly low (755 cc or about 16 cc per kg). The total blood volume was 4,100 cc, or about 86 cc per kg, which is within normal limits.

In the present investigation, 4 cases of morbus caeruleus have been studied with respect to total blood volume, circulating red cell volume and plasma volume. The subjects were adults, 3 males and one female, all of whom had veno-arterial shunts as evidenced by the marked cyanosis. Their cardiac status was carefully studied by physical examination and by fluoroscopic and electrocardiographic examinations of the heart. The oxygen content and capacity were determined by arterial puncture and in every instance oxygen unsaturation was increased. The "Evans Blue" dye was employed in determining plasma volume following the method described by Gibson.⁹ The dye concentration in the serum was determined with a Marten's spectrophotometer.

Results. It will be noted (Table I) that the hematocrit values are extremely high when it is remembered that the normal range is 45 to 50% for males and 40 to 45% for females. The highest value, 83%, recorded in this series was in a male of 18 years of age who was believed to have Tetralogy of Fallot. The lowest value, 72%, was obtained in a female 37 years of age who suffered from the same anomaly. The most striking feature (Table I) was the low absolute plasma volumes which were consistently found in all 4 cases. The lowest plasma volume (27.6 cc per kg) was obtained in the third case, a male 33 years of age. The average plasma volume in the 4 cases was 30 cc per kg.

The circulating red cell mass and total blood volume were found to be elevated in each instance.

In Table II we have compared the results obtained in morbus caeruleus with those of normal individuals and those with primary polycythemia (polycythemia vera). It will be noted that in morbus

⁷ Blumenfeldt, E., and Wolheim, E., *Klin. Wschr.*, 1927, **6**, 396.

⁸ Meyer, P., *Z. fur Klin. Med.*, 1932, **120**, 341.

⁹ Gibson, J. G., *J. Clin. Invest.*, 1937, **16**, 301.

TABLE I.
Blood Findings in Morbus Caeruleus (Cyanotic Type of Congenital Heart Disease).

K.C.	Age	Sex	Wt, kg	Hgb. % 17 g 100%	R.B.C. Hemato- crit per mm ³ (Mill.) (Vol.%)	Blood volume					Blood vol. per kg			% deviation from normal volume				
						Plasma vol. liters	Red cell vol. liters	Total Blood vol. liters	Plasma vol. cc	Red cell vol. cc	Total vol. cc	Plasma	Red cell	Total	Plasma	Red cell	Total	
K.C.	18	M	60.0	126	9.04	83	1.77	8.63	10.4	29.5	144.0	173	-36	+230	+90			
A.N.	37	F	67.5	133	10.8	72	1.98	5.02	7.0	29.3	74.5	104	-25	+100	+46			
I.W.B.	33	M	59.8	128	6.8	74	1.65	4.95	6.6	27.6	82.8	110	-41	+92	+22			
H.F.	43	M	45.5	137	6.5	73	1.54	4.16	5.7	33.9	91.5	125	-28	+110	+39			
										30.0	98.2	128						
										avg	avg	avg						

K.C. — Tetrolony of Folio-4

K.C. = Tetralogy of Fallot.

A.N. = " "

I.W.B. = Patent ductus arteriosus with patent interventricular septal defect.

H.F. = Infundibular stenosis with interventricular septal defect.

TABLE II.
Comparison of Blood Volume in Morbus Caeruleus (Cyanotic Type of Congenital Heart Disease) to That in Polycythemia Vera and in Normal Individuals.

	Plasma volume cc per kg	Red cell volume cc per kg	Total blood volume cc per kg
	Females		
Normal (avg of 10 normals)	39.0	32.0	70.8
Morbus caeruleus	29.3	74.5	104.0
Polycythemia vera (avg of 6 cases)	76.7	177.0	253.7
	Males		
Normal (avg of 10 normals)	46.4	43.1	89.5
Morbus caeruleus (avg of 3 cases)	30.3	109.0	136.0
Polycythemia vera (avg of 6 cases)	51.2	145.0	196.2

caeruleus the plasma volume is 29.3 cc per kg as compared to the mean normal of 39 cc per kg for females and 30.3 cc per kg as compared to a mean normal of 46.4 cc per kg for males. This represents a subnormal diminution of plasma volume from the mean normal by 26 and 35% respectively. When the plasma volume of morbus caeruleus is compared with that of polycythemia, it will be noted that the latter shows a definite increase even over the normal.

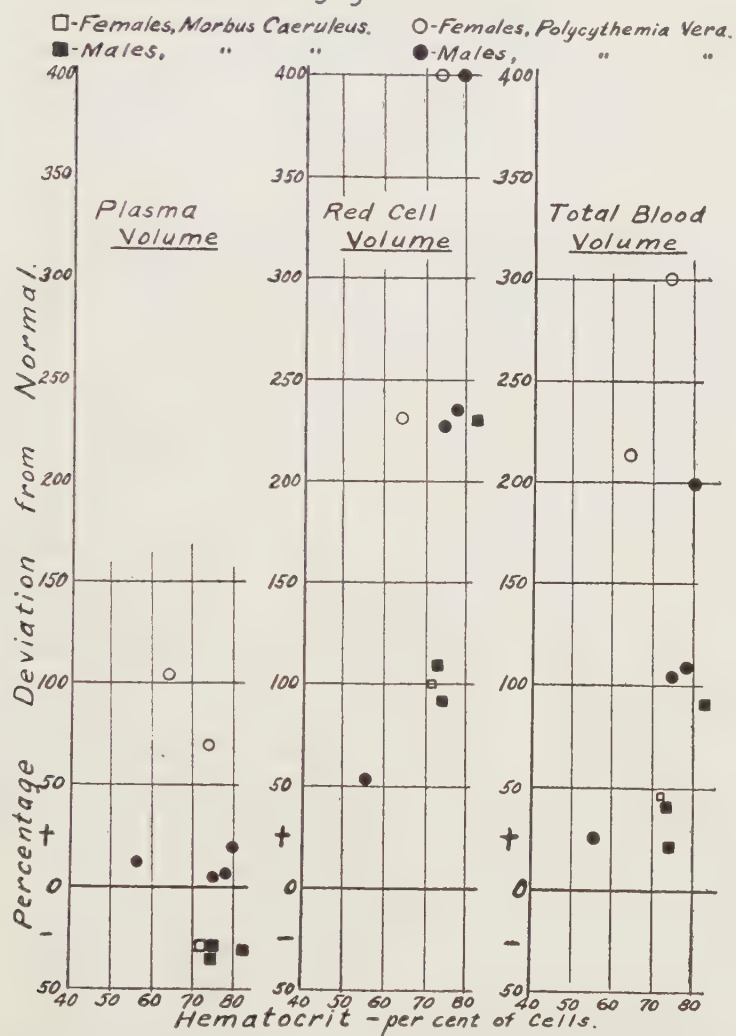
When the circulating red cell volume of morbus caeruleus is compared with the normal and with that in polycythemia vera (Table II) it is noted that for both males and females the red cell volume is decidedly increased in morbus caeruleus and strikingly increased in polycythemia vera.

The total blood volume is but moderately increased in morbus caeruleus while in polycythemia vera it is greatly increased. In one instance the increase was 300% (Fig. 1). This patient had a total blood volume of 18.28 liters of blood, approximately 3 times greater than normal.

In Fig. 1 it is noted that the plasma volumes of morbus caeruleus are all subnormal, a percentage deviation of -36%, -25%, -41%, and -28% respectively below the mean normal.

Summary. The results of the present study indicate that the plasma volume is subnormal in adult cases of morbus caeruleus while the total circulating blood volume is moderately increased, this increase being due to a considerable increase in circulating red cell volume. From the viewpoint of the disturbed dynamics of the circulation in morbus caeruleus, these blood volume findings would appear to indicate a compensatory effort on the part of the circulation to maintain a normal blood volume at the expense of a reduced plasma volume. At the same time, this permits the increase in circulating red cell volume which is necessary to compensate in part at least for the anoxia.

Fig. I. Comparison of Blood Volume in Morbus Caeruleus to that in Polycythemia Vera.



The blood volume findings in morbus caeruleus resemble those of polycythemia vera in that in both conditions the circulating red cell volume and total volume are increased both in relative and absolute values. The only difference is associated with the plasma volume change; in morbus caeruleus it is subnormal while in polycythemia vera the plasma volume tends to be slightly above normal.*

* Studies in two of the above cases were made possible through the courtesy of Dr. O. N. Nelson of the Minneapolis General Hospital.

11341 P

Effect of Epinephrine on Potassium Balance in the Perfused Hind Limbs of the Frog.

J. CLIFFORD STICKNEY. (Introduced by Ancel Keys.)

From the Laboratory of Physiological Hygiene, University of Minnesota Medical School, Minneapolis, Minn.

Intravenous injection of epinephrine produces an immediate transitory rise in the level of plasma potassium.^{1, 2, 3} The duration and magnitude of this effect differs in different animal species and a subsequent fall in [K]s to below normal is equally marked and less transitory, especially in man.⁴ The initial rise in [K]s seems to originate in the liver² but it appeared possible that the skeletal muscle might be involved in the slower and more sustained decline.

Perfusion preparations of the isolated hind limbs of the double-pithed frog were made. The perfusion fluid was a 3% gum acacia solution with the salt content and pH adjusted to correspond with normal frog Ringer's solution except for K which was somewhat high (5.18 to 6.03 m.eq./l). A perfusion pump supplied pulsating pressure to the inflow cannula entering the terminal aorta. The outflow was collected from cannulae in the renal portal veins, all other egress being prevented by ligatures. [K] was determined⁵ in arterial and venous samples collected at intervals during a period of 2½ to 3½ hours. Rates of flow were measured throughout.

In preliminary experiments single injection of epinephrine into the arterial inflow gave somewhat inconstant but essentially negative results with regard to the [K] in the venous outflow. In all cases the [K] in the arterial inflow was the most important factor in determining the direction and rate of K exchange between the tissue and the perfusion fluid. When epinephrine was continuously infused into the arterial inflow (0.005 mg/cc) there was consistently a marked effect on the arteriovenous K difference, but almost no effect on the rate of K exchange between the tissue and the perfusing fluid. In other words, in these experiments, the movement of K from vascular bed to tissue proceeded at a rate independent of the total rate

¹ D'Silva, J. L., *J. Physiol.*, 1934, **82**, 393.

² Marenzi, A. D., and Gerschman, R., *Rev. Soc. argent. de biol.*, 1936, **12**, 424.

³ Brewer, G., Larson, P. S., and Schroeder, A. R., *Am. J. Physiol.*, 1939, **126**, 708.

⁴ Keys, Ancel, *Am. J. Physiol.*, 1938, **121**, 325.

⁵ Hartzler, E. R., *J. Biol. Chem.*, 1937, **122**, 19.

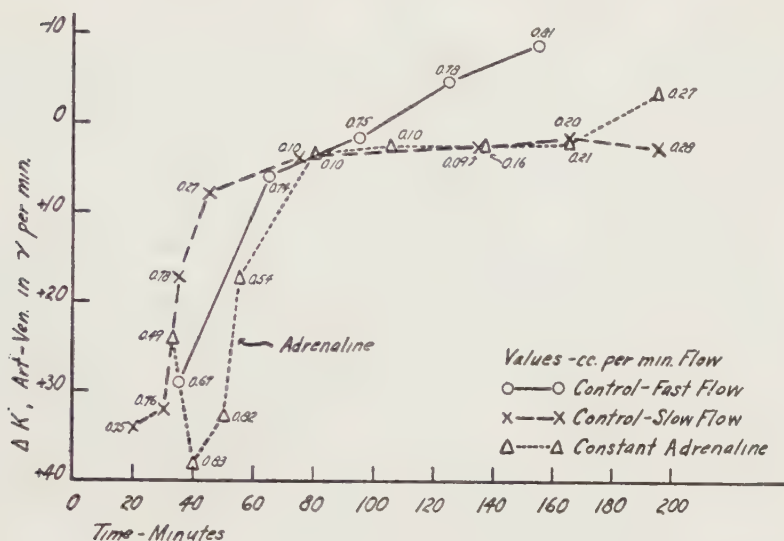


FIG. 1.

of flow. This was true whether the rate of flow was altered by epinephrine administration or by simple change in the perfusing pressure which was studied in separate experiments. These points are shown in Fig. 1 in which typical results are plotted.

These results show that when a small K gradient from blood vessel to tissue is applied in resting muscle, the rate of renewal of the blood phase is not, within physiological limits, a limiting factor for the K exchange, nor could a direct effect of epinephrine be seen. Further experiments with no net movement of K in control periods likewise failed to demonstrate a direct effect of epinephrine on K exchange or balance in resting muscle.

Elimination of Radioactive Elements in Patients Who Have Received Thorotrast Intravenously.

WILHELM STENSTROM AND IRWIN VIGNESS.

From the Department of Radiology, University of Minnesota.

The radioactive method of determining the elimination of any elements in the thorium series is more sensitive than the chemical method and has been used exclusively in this study which deals with the elimination from patients who previously have received intravenous injection of thorotrast. Most of the ionization produced is caused by α -particles and preliminary studies indicated that an electroscope was the most suitable instrument for the detection of the ionization. The limit of the sensitivity of the method used was such as to detect 0.005 cc of thorotrast mixed with 4 g of ash. The γ -ray Geiger-Müller counter did not give a reliable response to less than 1 cc of thorotrast at 1 cm distance.

Apparatus and Method. A Wulf bifilar type of electrometer, which has a low capacity, was used in conjunction with a cylindrical ionization chamber 18 cm in diameter and 26 cm long. The chamber was attached directly to the electrometer.

In order to obtain a measure of the thoron in the breath, a patient exhaled through a rubber tube into a closed cylindrical metal container having a diameter of 30 cm and a height of 30 cm. Several small holes in the cover provided for the escape of the exhaust air. A copper wire with an active length of 21 cm extended along the axis of the container. It was insulated from the grounded container and kept at a negative potential of about 2000 volts and served to collect the radioactive deposit caused by the disintegration of the thoron. After the collection had been made for a time, the wire was transferred and used as the central collecting electrode of the ionization chamber on top of the electrometer. The lengths of time that the patients breathed into the container were between 1 and 2 hours, but more deposit would build up if this could be continued for longer time (up to 20 or 30 consecutive hours). The half life time of thoron is only 55 seconds and that of thorium A 0.1 second. The next element, thorium B, has, however, a half life time of 10.6 hours and will therefore make the wire active for a relatively long time after it has been deposited.

The stool was collected in a waxed cardboard container. This box with its content was placed in an iron (sand bath) dish and heated

for about 5 hours until a light colored ash remained, usually weighing from 2 to 4 g. The ash was then spread evenly over a circular, light cardboard paper about 17 cm in diameter and having a central hole 4 cm in diameter.

The ash obtained from the urine was a heavy black fusible material which probably consisted of carbon contained in various salts. This ash was so bulky and heavy that the α -rays were largely absorbed and the unsatisfactory results obtained indicated that it will be necessary to extract the radioactive substances from the urine when the studies are continued.

Results. In order to obtain an idea of the sensitivity of the method, small amounts of thorotrast were mixed with 4 g of ash which then was heated and thoroughly stirred. Measurements of the radioactivity of ash containing different amounts of thorotrast are reproduced graphically in Fig. 1, where the discharge of the electroscope in scale divisions per minute is plotted against the known amount of thorotrast present in the ash. The 3 dots correspond to 0.01, 0.02, and 0.03 cc of thorotrast respectively.

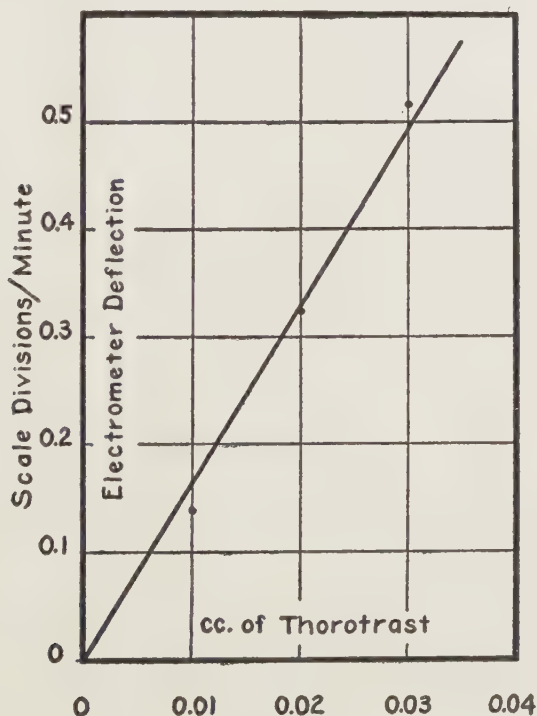


FIG. 1.

Examinations were made of the ashed feces from 2 patients who had intravenous injection of thorotrast 6 and 7 years previously. Each one had been given 75 cc of thorotrast which contained 24 to 26% thorium dioxide by volume. Easily measurable radioactivity was found in all the samples. When the samples were reexamined several weeks later, it was, however, found that the radioactivity had almost completely disappeared. It was, therefore, assumed that most of the radioactivity was due to elements with short lifetime belonging to the thorium series. A stool obtained from the second patient at a known time was then ashed as soon as possible and its activity studied as a function of time. The results are plotted in Fig. 2. The points represent the number of scale divisions the electroscope was discharged per minute at different hours from the time the stool was collected. Curves A, B, and C were calculated from the known

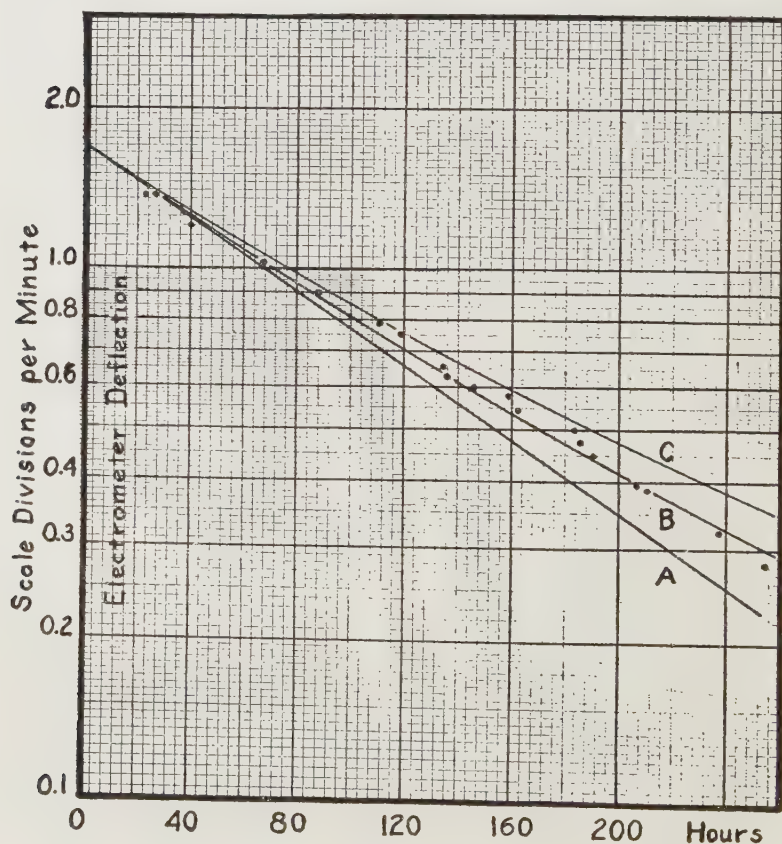


FIG. 2.

disintegration constants. Curve A shows the relation between radioactivity and time if only thorium X (half life time 87.4 hours) in equilibrium with its disintegration products was present, curve B shows the decay of a mixture of 95% thorium X and 5% of some preceding long lived product of the thorium series (thorium, mesothorium or radiothorium), and curve C illustrates a mixture of 91% thorium X and 9% long lived element. The radioactive elements present in the feces in this case seem to consist of approximately 95% thorium X and 5% of long lived elements, each in equilibrium with its disintegration products. A slight change in the normal background drift of the electrometer could, however, account for the difference between the curve representing thorium X and the experimental points. Measurements 4 months later indicate that 2 to 3% of the activity was due to long lived elements in equilibrium with their disintegration products.

Several samples of ashed feces from other individuals, who had never had any thorotrast injected, were also examined but in no case was any radioactivity discovered.

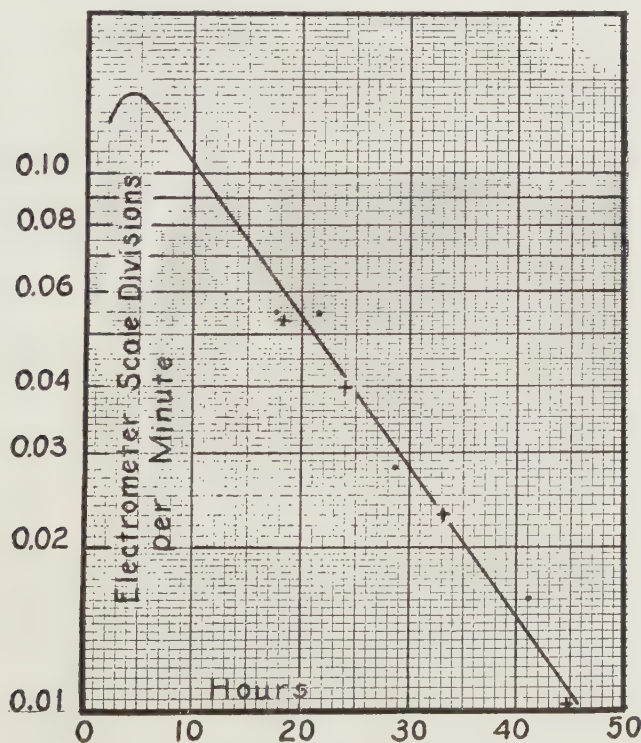


FIG. 3.

22 RADIOACTIVE ELEMENTS ELIMINATED AFTER THOROTRAST

One sample of ashed urine from the second patient showed slight radioactivity which decayed with time. The patients were discharged before urine could be obtained for an attempt to extract the radioactive substances.

No radioactivity was discovered in one sample of sputum which had been collected over a period of 24 hours and then ashed. More sputum examinations ought to be done.

The results from one of the breathing experiments are shown in Fig. 3. The points fall very close to the theoretical curve for disintegration of thorium B and it is, therefore, evident that a fair amount of thoron is exhaled by the patient. This must mean that the thoron can pass rapidly from the source (probably liver and spleen) to the breath as the half life time for the thoron is only 55 seconds.

Summary. No excretion of thorium has thus far been discovered. Certain radioactive elements in the thorium series have, however, been found in the feces, urine and breath. Thorium X has been identified as the predominant element excreted in the feces, and thoron is definitely exhaled, as thorium B has been identified in the radioactive deposit from the breath. Such excretion of thorium X and thoron leads to reduced radioactivity in the body even if the thorium itself remains. As most of the γ -rays are emitted by disintegration products of thoron it is evident that the amount of thorium remaining in the tissues of a patient can not be determined with satisfactory accuracy by measurements of the γ -rays emitted from the patient (liver and spleen). During the disintegration process of some of the radioactive atoms such a displacement must take place that the newly formed atoms can escape.

11343 P

Influence of Pregnancy and Lactation on Susceptibility to Arrest of Brain Circulation.*†

HERMAN KABAT. (Introduced by M. B. Visscher.)

From the University of Minnesota, Minneapolis.

By means of a new technic,¹ it has been possible to obtain constant results from dog to dog with the same period of complete arrest of the brain circulation. The sex, age, or breed of the experimental adult animal had no influence on the sensitivity of the brain to arrest of its blood flow. On the other hand, puppies were much more resistant than adult animals to this procedure.² The present report deals with the influence of pregnancy and lactation on the resistance of the brain to arrest of its circulation.

In every instance, the pregnant or lactating animal was more severely affected by brain stasis than the normal adult animal. A similar increase in susceptibility was shown by 2 young females at about the age of sexual maturity, one of whom was in the first oestrus. The results are compiled in Table I. Only the dogs indicated by asterisks (after survival) died from the arrest of the brain circulation. The others were either sacrificed or are still alive. The striking increase in severity of the brain damage as compared to normal adult animals is evident.

A study of the time of persistence of respiration and the corneal reflex following acute arrest of blood flow in the brain shows no difference between pregnant or lactating and normal adults. Furthermore, the recovery times of respiration and the corneal reflex following restoration of cephalic blood flow were very similar in the 2 groups of dogs. In other words, the survival time and the recovery time are within normal limits while the revival time³ is greatly decreased by pregnancy and lactation. This suggests the possibility that the greater susceptibility during pregnancy and lactation may be due, not to a difference in the rate of metabolism, but rather to a difference in the ability of the animal to overcome reversible neuronal damage.

* Aided by a grant from the Committee on Scientific Research of the American Medical Association.

† Assistance in the preparation of these materials was furnished by the personnel of Works Progress Administration, Official Project No. 665-71-3-69, sub-project No. 309.

¹ Kabat, H., and Dennis, C., *PROC. SOC. EXP. BIOL. AND MED.*, 1938, **38**, 864.

² Kabat, H., and Dennis, C., *PROC. SOC. EXP. BIOL. AND MED.*, 1939, **42**, 534.

³ Gerard, R. W., *Arch. Neurol. and Psychiat.*, 1938, **40**, 985.

LACTATION: SUSCEPTIBILITY TO BRAIN ANEMIA

TABLE I.
Influence of Pregnancy and Lactation on Susceptibility to Arrest of the Brain Circulation.

Dog	Sex	Condition	Period of stasis, min	Recovers ability to sit up	Recovers ability to stand and walk	Duration of coma	Duration of apathy, days	Duration of ataxia	Survival	End result and comment
BK14	M	Normal adult	2	12 hr	18 hr	12-18 hr	2	1 wk	4 mo	Apparently complete recovery
BK22	F	" "	2	12 "	18 "	12-18 "	2	5 days	4 "	" "
18	F	Late pregnancy	2	1 day	9 days	12-18 "	9	2 mo	2½ "	" "
BK16	F	Active lactation	2	No recovery	No recovery	As long as she survived	—	—	3 days*	No recovery from coma
17	F	Normal adult	4	18 hr	18 hr	18 hr	4	19 days	19 "	Apparently complete recovery except for slight ataxia
BK3	M	" "	4	1 day	2 days	18 "	4	21 "	3 mo	Apparently complete recovery
BK17	M	" "	4	1 "	1½ "	24 "	3	18 "	4 "	" "
BK13	F	Active lactation	4	No recovery	No recovery	As long as she survived	—	—	6 days	No recovery from coma
La 1	F	Late lactation (pups weaned)	4	1 day	8 days	24 hr	14	39 days	3 mo	Apparently complete recovery
La 2	F	Active lactation	5	6 "	12 "	12 days	20	Still has clumsy gait	Still alive (4 mo)	Permanent clumsy gait, slow movements, little spontaneous activity, little emotional expression
BK15	F	Normal adult	6	24 hr	40 hr	40 hr	4	1½ mos	Still alive (9 mo)	Apparently complete recovery
BK20	M	" "	6	24 "	48 "	24 "	3	3 wk	3 mo	" "
BK21	F	" "	6	24 "	48 "	24 "	3	3 "	3 "	" "
BK18	F	Young ♀ reaching sex maturity	6	No recovery	No recovery	As long as she survived	—	—	9 days	Littermate of BK20 No recovery from coma

Inheritance of Chlorophyll in F_1 Crosses Made Reciprocally Between Selfed Lines of Corn.*

ELMER S. MILLER AND I. J. JOHNSON.†

From the Department of Botany, Agronomy and Plant Genetics, University of Minnesota, Minneapolis.

The inheritance of chlorophyll variations in plants has been investigated in detail; over one hundred chlorophyll abnormalities have been reported in Maize. Correns¹ suggested that chlorophyll deficiencies were transmitted as inclusions and plastids through the cytoplasm of the female parent during fertilization, but that none was transmitted from the male parent in the cytoplasm, along with the male nucleus to the egg. The classical example is the transmission of variegation in *Mirabilis* only through the cytoplasm of the egg, but not along with the male nucleus. These findings have been confirmed by Baur² and are supported by East.³

Recently Anderson⁴ has reported the results of a series of experiments and he concludes:

- a. Plastids are identical and numerous in both female and male gametophytes.
- b. In the pollen tube, numerous plastids were always present near the male nuclei, and as the tube is ruptured, the plastids are expelled into the cytoplasm of the embryo sac.
- c. The earlier investigators fixed and preserved samples in such a manner that all the inclusions were dissolved.

In this study, the writers have on a physiological basis set up the following experiment to further determine whether maternal inheritance, as transmitted by cytoplasm of respective parent to F_1 , is of any importance in inheritance studies of the chlorophyll pigments.

* Contribution from the Department of Botany and Division of Agronomy and Plant Genetics, University of Minnesota. Paper No. 1776 of the Journal Series. Aided by a grant from the Graduate School of the University of Minnesota. Assistance in the preparation of these materials was furnished by the personnel of Works Projects Administration Official Project No. 65-1-71-140, Sub-project No. 331.

† Now Professor and Research Professor of Farm Crops, Iowa State College, Ames, Iowa. Formerly in Agronomy and Plant Genetics, University Farm.

¹ Correns, G., *Z. Ind. Abst. Vererb.*, 1909, **1**, 291.

² Baur, E., *Z. Ind. Abst. Vererb.*, 1909, **1**, 330.

³ East, E. M., *Am. Nat.*, 1934, **63**, 289; 402.

⁴ Anderson, Lewis F., *Am. J. Bot.*, 1936, **23**, 490.

The method of sampling, storing, extracting, and quantitative analysis has previously been described by Miller and Johnson.⁵

The analytical data are presented in Table I of total chlorophyll concentration expressed as percent on a green weight basis and as milligrams per 100 sq cm of leaf surface in 15 crosses between high chlorophyll female X low male and reciprocally in low chlorophyll female high male. The values reported are the average of 2 field replications grown in 1939. The inbred parents used in these crosses differed significantly in percent total chlorophyll; the highest chlorophyll inbred parent of the low chlorophyll lines being significantly lower than the lowest parent in the high chlorophyll lines as measured by the analysis of variance in 2 field replications in 1938 and 1939.

The transmission of variegation (unequal distribution of chlorophyll in the leaves) in *Mirabilis* occurs only through the egg cell cytoplasm. This has raised the question, whether any plastids are transmitted in the cytoplasm contributed by the male parent; and if there are, of what importance is such a transmission, in comparison with that contributed by the female.

During fertilization, according to Anderson, it is possible for plastids in the cytoplasm to be carried along with the male nucleus when the pollen tube ruptures. In *Antirrhinum*, during the time that the male nucleus migrates through the cytoplasm of the embryo

TABLE I.
% Total Chlorophyll and mg of Total Chlorophyll per 100 cm² of Leaf Tissue in
Reciprocal Crosses Between High and Low Chlorophyll Inbred Lines of Corn.

Cross	Inbred parents	% total chlorophyll F ₁ crosses				Mg chlorophyll per 100 cm ² F ₁ crosses			
		H x L	L x H	Dif.		H x L	L x H	Dif.	
6 x 23	.319 x .352	.367	.286	.81		7.12	5.78	1.34	
x 26	x .360	.305	.289	.16		6.64	6.20	.44	
x 44	x .361	.360	.357	.03		6.82	6.63	.19	
x 48	x .364	.321	.328	-.07		6.28	6.70	-.42	
11 x 23	.232 x .352	.299	.296	.03		6.16	6.01	.15	
x 26	x .360	.274	.279	-.05		6.00	6.30	-.30	
x 44	x .361	.330	.342	-.12		6.52	7.06	-.54	
x 48	x .364	.288	.319	-.31		5.93	6.51	-.58	
34 x 23	.273 x .352	.331	.326	.05		7.04	6.66	.38	
x 26	x .360	.322	.282	.40		7.50	6.63	.87	
x 48	x .364	.299	.299	.00		6.53	6.45	.08	
45 x 23	.306 x .352	.342	.356	-.14		6.51	6.56	-.05	
x 26	x .360	.299	.313	-.14		6.15	6.44	-.29	
x 44	x .361	.361	.356	.05		6.50	7.03	-.53	
x 48	x .364	.386	.375	.11		6.93	6.93	.00	
Mean		.326	.320	.05400		6.57	6.52	.049333	
		Z = .21	Odds = 3.4:1			Z = .093	Odds = 1.7:1		

⁵ Miller, Elmer S., and Johnson, I. J., *Am. Soc. Agron.*, 1938, **30**, 941.

sac, and while the male nucleus is in the process of fusing with the polar nuclei to form the endosperm nucleus, there are mitochondria in the vicinity of the endosperm nucleus. These mitochondria originally came from both the male and female parents.

Thus, on a cytological basis, it appears that there are species differences regarding the mechanism and importance of cytoplasmic inheritance. In *Mirabilis*, it is important with respect to leaf variegation, but in other cases, *i. e.*, corn, with odds as low as 3.4 to 1 and 1.7 to 1 for percent total chlorophyll and milligrams chlorophyll per 100 sq cm leaf surface respectively, as measured by "Students" pairing method and tables of Z, it is apparent that cytoplasmic inheritance is not an important factor in inheritance studies of chlorophyll in corn.

Summary. These studies show that maternal inheritance of chlorophylls as measured by a series of crosses made reciprocally between high and low chlorophyll inbred lines of corn is not significant, and that male and female parents each contribute equally to the genotype of the F_1 cross in respect to chlorophyll concentration.

11345

In vitro Experiments on Exchange of Phosphate by Enamel and Dentin.

W. D. ARMSTRONG. (Introduced by F. H. Scott.)

From the Laboratories of Dental Research and Physiological Chemistry, University of Minnesota, Minneapolis, Minn.

Krogh, Holst, and Hevesy,¹ and Manly and Bale² demonstrated the presence of radioactive phosphorus in the whole teeth of animals receiving a parenteral administration of compounds of this isotope. Hevesy and Armstrong³ in an investigation in which the enamel and dentin of cats' teeth were separately studied found, using radioactive phosphorus as an indicator, the rate of exchange of phosphate by the enamel of erupted mature teeth to be about one-tenth that of the dentin. The rate of exchange was such as to make highly improbable an ability of enamel of mature teeth to undergo significant changes of composition as a result of nutritional alterations.

¹ Krogh, A., Holst, J. J., and Hevesy, G., *Det. Kgl. Danske Vidensk. Selskab. Biol. Med.*, 1937, **13**, 13.

² Manly, L., and Bale, W. F., *J. Biol. Chem.*, 1939, **129**, 125.

³ Hevesy, G., and Armstrong, W. D., *Proc. Am. Soc. Biol. Chem.*, 1940, XLIV.

The experiments herewith reported were carried out by agitating 0.12-0.15 mg of the finely pulverized specimens in 10 cc volumetric flasks completely filled with the solution of labeled phosphate. The active material was supplied by the Radiation Laboratory of the Department of Physics of this University. The specimens after the stated time of contact with the active solution were recovered and washed ten times with water by centrifugation.

These results obtained *in vitro*, especially in the case of dentin, show a surprisingly rapid rate of exchange of phosphate. However, the relative rates of exchange by enamel and dentin are very similar to those observed *in vivo* by Hevesy and Armstrong.³

Since dentin contains⁴ 22.2% protein and enamel less than 1% of protein, the larger amount of labeled phosphate acquired by dentin from solutions at pH 7.5 might have been due, if the protein of dentin has an isoelectric point somewhat above pH 7.5, to combination of phosphate anion with dentin protein. The acid combining power of dentin protein in relation to hydrogen ion concentration is not known but it appears very unlikely that this protein could combine with anions in solutions of pH 13. The experiments whose results are tabulated in the second and third columns of Table I indicate almost identical rates of acquisition of labeled phosphate by dentin from a solution of pH 7.5 and 13. It is, therefore, unlikely that the higher rate of uptake of radioactive phosphate by dentin at pH 7.5 was due to chemical combination of phosphate with dentin protein. The 2 experiments with dentin at pH 7.5 and 13 also indicate no effect of the state of ionization of phosphate on its rate of exchange with dentin. •

TABLE I.
Exchange of Phosphorus by Enamel and Dentin.

Material	Enamel	Dentin	Dentin	Dentin Protein
pH solution	7.42	7.51	13	7.4
Time in hr at 38°	20	20	22.5	20
Activity (counts/min)	19.6 ±0.33	111.0 ±1.6	218 ±1.9	8.85±0.23
Background (counts/min)	8.62±0.21	8.62±0.21	8.80±0.17	8.80±0.17
Mg P in solution	18.5	18.5	18.5	14.0
Mg P labeled by 1 count*	0.0122	0.0122	0.0114	
Mg P exchanged/g/24 hr	1.42	11.7	11.8	
% P exchanged/24 hr	0.81	9.19	9.26	
Specific activity†	0.0445	0.502	0.505	
Relative specific activity	1	11.2	11.3	

* Calculated from total activity of solution and its content of phosphorus.

† Specific activity: The % of total activity in a tissue per mg of phosphorus.

⁴ Armstrong, W. D., Brekhuis, P. J., and Cavett, J. W., *J. Dent. Research*, 1936,

As further evidence that dentin protein does not combine with phosphate at pH 7.5, denatured dentin protein⁴ was agitated for 20 hours with a solution of labeled phosphate with the result that no active phosphate was present in the protein after thorough washing with water.

The greater rate of exchange of phosphate by dentin must, therefore, be attributed to the smaller size of the crystallites of the mineral phase of dentin, but more especially to the fact that dentin is permeated by the dentinal tubules which probably have the effect of permitting a more complete contact of the crystals of the mineral phase with the solution containing labeled phosphate than exists in the case of enamel. An alternative hypothesis might be that the higher activity of dentin is due merely to some active solution trapped in the dentinal tubules. Nevertheless, phosphate in solution which is not removed from the tubules by thorough washing would be expected, with time, to reach an exchange equilibrium with the phosphate of the mineral phase.

11346

Cinnamic Acid Metabolism in Man.

I. SNAPPER, T. F. YÜ AND Y. T. CHIANG. (Introduced by S. H. Liu.)

From the Department of Medicine, Peiping Union Medical College, Peiping, China.

The fate of cinnamic acid ($C_6H_5CH:CHCOOH$) after its administration to the animal organism has been examined repeatedly. The following facts are known. Cinnamic acid given by mouth to humans is oxidized to benzoic acid which is excreted in the urine linked with glycine in the form of hippuric acid.^{1, 2} In cats and dogs after the administration of phenylpropionic acid not only hippuric acid but also small amounts of cinnamoylglycine are excreted.³ After administration of cinnamic acid hippuric acid with small traces of cinnamoylglycine are found.⁴ After administration of cinnamic

¹ Erdmann and Marchand, *Liebig's Annalen der Chemie und Pharmacie*, 1842, **44**, 344.

² Knoop, F., *Beiträge zur Chemischen Physiologie*, 1905, **6**, 150, and 1908, **11**, 411.

³ Dakin, H. D., *J. Biol. Chem.*, 1906, **5**, 173, 303.

⁴ Dakin, H. D., *J. Biol. Chem.*, 1907, **6**, 203.

acid by mouth to dogs 65-70% of the excreted benzoic acid is present in the form of benzoylglycuronic acid and 30% in the form of hippuric acid.⁵ The isolated kidney of calves and sheep is able to oxidize cinnamic acid to benzoic acid during perfusion.⁶ However, the isolated kidney of the dog is not able to oxidize cinnamic acid but only conjugates the cinnamic acid with glycine to form cinnamoylglycine.⁷ Excretion of cinnamoylglycuronic acid has not been observed previously. We have reexamined the question whether after cinnamic acid administration to humans free or conjugated cinnamic acid could be found in the urine.

Experimental. Six g of cinnamic acid were given after dissolving in 200 cc of water and neutralizing. The urine excreted within the first 4 hours of the experiment was examined in order to compare the results obtained with Quick's hippuric acid test.

1. *Glycuronic acid* was excreted in considerable amounts. The urine always gave a positive naphthoresorcin reaction.⁸ The amount of glycuronic acid varied between 300 and 800 mg determined with the Shaffer-Hartmann technic.⁹

2. *Hippuric acid.* To the 4 hours' urine concentrated HCl was added until the reaction became acid to Congo red. A considerable precipitate (4-5 g) was formed consisting of hippuric acid. After standing in the icebox for some hours the precipitate was filtered off. The filtrate still contained about 0.330 g of hippuric acid per 100 cc.

3. *Presence of free cinnamic acid.* Only small amounts of free cinnamic acid were excreted in this experiment. After chloroform extraction of the acidified urine, 10-15 mg of free cinnamic acid were demonstrated in the extraction fluid.

4. *Presence of a cinnamic acid compound.* Concentrated NaOH was added to the urine filtrate until the reaction was frankly alkaline. The alkaline fluid was boiled for one hour over a free flame and was then evaporated on a waterbath to a volume of 10-30 cc. After cooling, strong HCl was added. As soon as the reaction became acid, a thick white precipitate formed. After a few hours in the icebox, this precipitate was filtered off and dried in an incubator overnight. Next morning the precipitate was extracted on the waterbath for 2 hours with 150 cc of chloroform under a reflux con-

⁵ Quick, A. J., *J. Biol. Chem.*, 1928, **77**, 581.

⁶ Snapper, I., and Grünbaum, A., *Bioch. Z.*, 1924, **150**, 12.

⁷ Snapper, I., and Grünbaum, A., *Acta brevia Neerlandica*, 1934, **4**, 38, and *Pharmaceutisch Weekblad*, 1934, Jubileumboek Prof. P. van der Wielen.

⁸ Tollens, B., Allen's comm. Organic Analysis, 5th edition, **1**, 496, and *Ber. d. Deutsch. chem. Ges.*, 1908, **41**, 1788.

⁹ Quick, A. J., *J. Biol. Chem.*, 1926, **69**, 555.

denser. The chloroform was then filtered into a separatory funnel and extracted twice with 5 cc 8% NaOH.

To this alkaline solution, concentrated HCl was added until precipitation occurred. The precipitate was filtered off and dried. This precipitate contained a considerable amount of cinnamic acid. A. If, to a few mg of these crystals, one drop of Na_2CO_3 and one drop of KMnO_4 solution were added, the KMnO_4 quickly turned brown by reduction and a strong smell of benzaldehyde developed. B. By titration with bromine, considerable amounts of cinnamic acid were determined. For this titration a combination of the methods published by A. W. K. de Jong¹⁰ and by Greenberg and Mackay¹¹ was used. C. Pure cinnamic acid crystals with a melting point of 133°C were recovered by recrystallization from boiling petroleum ether.

5. *Presence of cinnamic acid as monocinnamoylglycuronic acid.* As the cinnamic acid compound present in the urine was very soluble in water, the possibility of the presence of cinnamic acid conjugated with glycuronic acid had to be considered. The following experiment makes the presence of a cinnamoylglycuronic acid compound seem probable.

The filtrate obtained after acidifying the urine with HCl was extracted with ether in a continuous Lind extractor for 15-20 hours. At that time the ether in the extraction flask showed a watery layer. This watery layer was separated from the ether and transferred to a 25 cc volumetric flask. After addition of 2 cc concentrated HCl, the volume was made up to 25 cc.

This solution was strongly reducing. Two samples of 4 cc each were used for the quantitative determination of glycuronic acid (Shaffer-Hartmann method). The remaining 17 cc were boiled for 30 minutes under a reflux condenser and then transferred while still hot to a separatory funnel in which 100 cc chloroform was already present. After cooling, the solution was shaken for 30 minutes. This was repeated twice with fresh chloroform. The 3 fractions of chloroform were mixed and shaken twice with 10 cc 8% NaOH. The NaOH was then made up to 25 cc in a volumetric flask. Two samples of 5 cc each were titrated with bromine. The remaining 15 cc were slightly concentrated by evaporation before a fan and then acidified with concentrated HCl. The precipitate was dried, weighed and titrated.

¹⁰ de Jong, A. W. K., *Am. J. Med. Sc.*, 1933, **185**, 630.

¹¹ Greenberg, D. M., and Mackey, M. A., *J. Biol. Chem.*, 1932, **96**, 419.

TABLE I.
Contents of Glycuronic Acid and Cinnamic Acid of the Water-soluble Fraction in the Extraction Flask of Lind Apparatus After Extraction for 20 hr.

	Glycuronic acid	Cinnamic acid	
	Found, mg	Found, mg	Calculated*
1	222	166.4	177.6
2	320	248.6	256
3	261	210	209
4	150.8	114.5	121.3
5	195.7	149	156
6	175.0	133	140

*From monocinnamoylglycuronic acid.

The glycuronic acid content of the 25 cc of watery solution was about equivalent to the amount of cinnamic acid found. When N mg glycuronic acid (M.W. 184) were found by reduction, the cinnamic acid content proved to be about $148/184 \times N$ mg (Table I).

The stoichiometric relation existing between glycuronic acid and cinnamic acid in the fluid analyzed is a strong indication that monocinnamoylglycuronic acid was excreted after administration of cinnamic acid to humans, especially if one takes into consideration that the cinnamic acid compound is water-soluble, insoluble in chloroform, hardly soluble in ether and is easily hydrolyzed by boiling with HCl or NaOH.

6. *Absence of cinnamoylglycine.* Cinnamoylglycine, being ether soluble, if present should have been found in the ether after extraction in the Lind apparatus. In order to test for the presence of cinnamoylglycine, the crystals obtained after the distillation of the ether were dissolved in boiling water and crystallized. The fraction which crystallized when the solution was still warm was separated and recrystallized from hot water. After purification the crystals did not give the cinnamic acid reaction with Na_2CO_3 and KMnO_4 .

Table II shows the excretion of hippuric acid in the four-hour period following the administration of cinnamic acid to humans. Six grams of cinnamic acid (equivalent to 5 g benzoic acid) brought about the excretion of 3.6-5.3 g of hippuric acid. These amounts are practically the same as those following the administration of equivalent amounts of benzoic acid.¹² These results indicate that the oxidation of cinnamic acid to benzoic acid occurs rapidly.

¹² Snapper, I., *Klinische Wochenschr.*, 1924, **3**, 56; Quick, A. J., *Am. J. Med. Sc.*, 1933, **185**, 630.

TABLE II.
Excretion within 4 hr.

Diagnosis	After 5 g benzoic acid Hippuric acid g	After 6 g cinnamic acid		
		Hippuric acid g	Cinnamic acid g	Glycuronic acid g
Sp. Reconvalescent Dysentery	5.00	4.31	0.216	0.596
L.S.S. Reconvalescent Diphtheria	4.32	3.60	0.294	0.458
F.L.Y. Healed Kala Azar	4.82	5.25	0.384	0.798
Y.H.S. Reconvalescent Lobar pneumonia	5.07	4.86	0.203	0.653
L.K.S. Reconvalescent Diphtheria	4.77	4.32	0.286	0.632
L.S.C. Avitaminosis A	5.12	4.72	0.202	0.635
C.H.C. Diencephalic Syndrome	5.89	5.28	0.367	0.654

During the same 4-hour period 400-800 mg of glycuronic acid were excreted. A small part of the cinnamic acid escapes β -oxidation. After giving 6 g of cinnamic acid to persons with normal liver function 200-400 mg of cinnamic acid, conjugated with glycuronic acid, were excreted within 4 hours (Table II).

Splenectomy and Benzol Injection as Means of Increasing Susceptibility of Chinese Hamsters to Kala-Azar.

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It has been shown in experimental animals that splenectomy and benzol injection frequently give rise to an increase of susceptibility of the animals to bacterial, parasitic, as well as virus infections.^{1, 2, 3} In parasitic infections such observations have been made with plasmodia, spirochetes and trypanosomes in monkeys, mice, rats, squirrels, etc. The effect of splenectomy and benzol injection on the susceptibility of the Chinese hamster, *Cricetulus griseus*, to *Leishmania donovani* has not been hitherto recorded. The following experiment was, therefore, designed, and the findings are here reported. The flagellate form of *Leishmania* was employed in the present study.

The experiment consisted of 3 groups of 20 hamsters each. The first group consisted of 20 splenectomized hamsters, the second, 20 benzol-injected hamsters and the third, 20 normal hamsters as controls. Splenectomy was carried out under ether anesthesia. All the splenectomized hamsters were allowed to recover from the effects of the operation for a period of from 1 to 2 months, before they were given the inoculation of flagellates. Of the 20 hamsters in the benzol group 10 were each injected subcutaneously with a single dose of 0.5 cc of a mixture of equal parts of benzol and sterile olive oil, 5 with 1 cc each, and another 5 with 2 cc each. The interval between the injection of benzol and inoculation of flagellates was 30 hours in the 0.5 cc group, 48 hours in the 1 cc group, and 7 days in the 2 cc group. From a preliminary determination it was found that the maximum tolerated single dose of the benzol mixture approaches closely to 2 cc for an average hamster. The inoculation of flagellates seems to be preferably given about 48 hours after the benzol injection. The flagellates used in the present study were prepared by pooling the condensation fluid of some 20 tubes of a 13-day culture which had been grown on NNN medium from the spleen emulsion of a heavily infected hamster. On May 10, 1939, each of the 60 hamsters in the 3 groups was given on the same day a single intraperi-

¹ Adler, S., *Trans. Roy. Soc. Trop. Med. and Hyg.*, 1930, **24**, 75.

² Meleney, H. E., *J. Exp. Med.*, 1928, **48**, 65.

³ Zinsser, H., and Castaneda, M. R., *J. Exp. Med.*, 1930, **52**, 649.

toneal inoculation of 0.2 cc of the culture, estimated to contain 145,000 flagellates.⁴

After the inoculation of flagellates infection in the hamsters was first determined by liver puncture and then by the examination of smears and sections made from the spleen and liver at autopsy. Two liver punctures were done before the hamsters were sacrificed for examination. Only 3 or 4 hamsters from each group were punctured. The first puncture was done 29 days and the second, 56 days after the infective inoculation. The results of the first puncture were all negative. But by the time of the second puncture 1 or 2 hamsters from each group already showed positive smears. In order to make use of the rate of early infection to determine the degree of susceptibility, all the hamsters which had survived up to the time of the second puncture were sacrificed. Not only were smears and sections made from the spleen and liver of the hamsters but also the weight of the hamsters as well as that of their spleen and liver were recorded. During the period of infection 2 hamsters from the splenectomized group died. Their organs were not suitable for examination because of marked post-mortem changes. The results of the examination of the spleen and liver of all the remaining hamsters are shown in Table I in which only the parasitological findings of sections were recorded, as they were found to be more conclusive than those afforded by direct examination of smears.

As shown in Table I, in the group of 18 splenectomized hamsters which were killed and examined at the conclusion of the experiment all except one showed a large number of parasites in the sections made from the liver. On the other hand, in the group of control hamsters, only 8 out of 18 (liver of 2 hamsters not examined) hamsters gave positive liver sections, in most of which only a few parasites were found, although positive spleen sections were seen in 14 of the 20 hamsters. In this group 6 hamsters failed to take the infection. In the benzol-injected group 4 hamsters showed negative spleen sections and 9 showed negative liver sections. Four hamsters in this group failed to contract kala-azar. There was no striking difference of the infection rate among the various dosage groups of benzol-injected hamsters. The 2 cc group seemed to have given the highest infection rate, but repeated smaller doses which were not tried in the present study, might give a higher infection rate.

Table II shows the average weight of the hamsters and that of their spleen and liver. In the benzol group the weighing of the

⁴ Earle, W. C., and Perez, M., *J. Lab. and Clin. Med.*, 1932, **17**, 1124.

TABLE I.
Parasitological Findings of Sections of Spleen and Liver of Splenectomized, Benzol-injected and Normal Hamsters.

Hamster No.	Splenectomized hamsters		Benzol-injected hamsters		Control hamsters	
	Hamster No.	Leishman-Donovan bodies in liver sections	Hamster No.	Leishman-Donovan bodies in sections of liver	Hamster No.	Leishman-Donovan bodies in sections of liver
3003	3035	++	3035	++	3045	(+)
3004	3036	+	3036	++	3046	0
3005	3037	died (not examined)	3037	++	3047	++
3006	3038	,,	3038	++	3048	0
3007	3039	++	3039	++	3049	0
3008	3040	(+)	3040	++	3050	0
3009	3041	++	3041	0	3051	++
3010	3042	0	3042	0	3052	0
3011	3043	++	3043	0	3053	++
3012	3044	++	3044	(+)	3054	0
3013	3070	++	3070	0	3082	0
3014	3071	++	3071	(+)	3083	0
3015	3072	++	3072	+	3084	(+)
3016	3073	++	3073	0	3085	(+)
3017	3074	++	3074	+	3086	++
3018	3065	++	3065	0	3087	++
3019	3066	++	3066	+	3088	++
3020	3067	++	3067	0	3089	0
3021	3068	++	3068	++	3090	(+)
3022	3069	++	3069	0	3091	(+)
Hamsters Nos. 3035-3044 incl. each received 0.5 cc of benzol mixture.						
,,	3070-3074	,,	1.0	,,	,,	,,
,,	3065-3069	,,	2.0	,,	,,	,,
- = Not examined.						
0 = Not found.						
(+) = Very few found.						
++ = Found in every 11-20 oil immersion fields.						
++	++	,,	6-10	,,	,,	,,
++	++	,,	1-5	,,	,,	,,
++	++	,,	,,	,,	,,	,,

TABLE II.
Average Weight of Body, Spleen and Liver of Splenectomized, Benzol-injected and Normal Hamsters.

	Body weight, g	Liver weight, g	Spleen weight, g
Control hamsters	26.1	1.023	0.089
Splenectomized hamsters	28.7	1.320	—
Benzol-injected hamsters	26.6	—	0.105

liver was not done. Taking the normal weight of the hamster's spleen as from 0.15 to 0.3% of the body weight⁵ it is evident that the average weight of the spleen in both benzol-injected and control hamsters was above normal. The increase of the spleen weight was apparently due to the kala-azar infection. The difference of the average spleen weight between these two groups seems to be out of proportion to the difference of their average body weight. As the rate of spleen infection was higher in the benzol-injected hamsters than in the controls, it appears likely that the greater average spleen weight of the benzol-injected group was due to the heavier infection rather than the greater average body weight. In the splenectomized hamsters the average liver weight was again greater than that of the controls. This was also likely due to the heavier infection rather than the greater average body weight of the splenectomized animals, although a part of the increased liver weight might have been due to a compensatory hyperplasia of the reticulo-endothelial system.

Conclusions. Chinese hamsters became more susceptible to infection with the flagellates of *Leishmania donovani* after splenectomy or benzol injection. Of the two means employed to increase the susceptibility of hamsters to kala-azar infection splenectomy appears to be more effective.

⁵ Meleney, H. E., *Am. J. Path.*, 1925, **1**, 147.

11348 P

Optic Nerve Response to Retinal Stimulation in the Rabbit.*

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With changes in intensity and duration of light, the retinal potential undergoes slight changes in form, while the nerve discharges show transformations corresponding to the form of the stimulus.

The fore part of the rabbit's brain was removed under ether, exposing the optic nerves. Records were taken from one nerve and from across the corresponding retina. Light intensities used were high but within the physiological range, as indicated by reduction of response with reduction of intensity. Experiments were conducted in a dark room, flashes being delivered at about 1 per second from a 2-mm slit in the lamp housing, in front of which a sector disk was rotated. A lens projected an image of the slit on the rabbit's cornea, the eye thus focused an image of the lens on the retina. This image stimulus was compared with one from a diffusing screen close to the cornea illuminated with a $1\frac{1}{4}$ -inch spot of light.

With flashes as short as 5 ms the retinal potential shows the usual *a* wave, a diphasic *b* wave, and no *c*. The "off" effect does not appear. With longer durations the *b* wave assumes its conventional

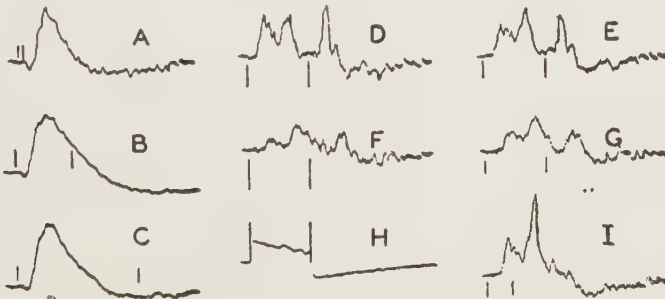


FIG. 1.

A, B, C, records from retina, light 18,000 candles per sq ft on 10 sq mm retinal image, durations of 7, 85, and 195 ms respectively, as marked. D.C. amplifier. Oculomotor nucleus destroyed after A. D, E, F, records from optic nerve, same stimulus, 101 ms duration, but with apertures at lens of 72, 30, and 8 mm diameter, ratios of 1, $1/6$, and $1/81$ areas of image. G, 72 mm aperture, intensity 1,800 candles per sq ft, or $1/10$ th the previous. This record falls between $1/6$ th and $1/81$ st aperture records in amplitude. H, photocell record. I, like D, shorter duration, 40 ms. The "off" spike is summed with the second "on" wave.

* Assisted by grants from the Rockefeller Foundation and from the Scottish Rite Masons' Fund.

monophasic form which does not return to the base line during illumination. (Fig. 1.) Between 5 and 200 ms duration, and between 18,000 and 200 candles per sq ft, the form of the retinal *b* wave changes surprisingly little.

On the contrary, the nerve discharge alters progressively over these ranges. The characteristic "on" response to a short bright flash occurs in two parts, the first consisting of a sharp initial spike followed usually by a decreasing series at 10-ms intervals, and the second, a rise which falls off during further illumination, its peak at about 30 ms after the first. The "off" response consists of a spike series similar to the first "on".

As *duration* is decreased, the "off" response decreases in amplitude, but is still distinct at 7 ms, while the "on" discharges decrease only at still shorter durations. As *intensity* is decreased, the "on" responses decrease in amplitude more rapidly than the "off", and the first "on" discharge more rapidly than the second, suggesting that different fibers are involved. The second "on" response and the "off" may sum quantitatively, again indicating different fibers in

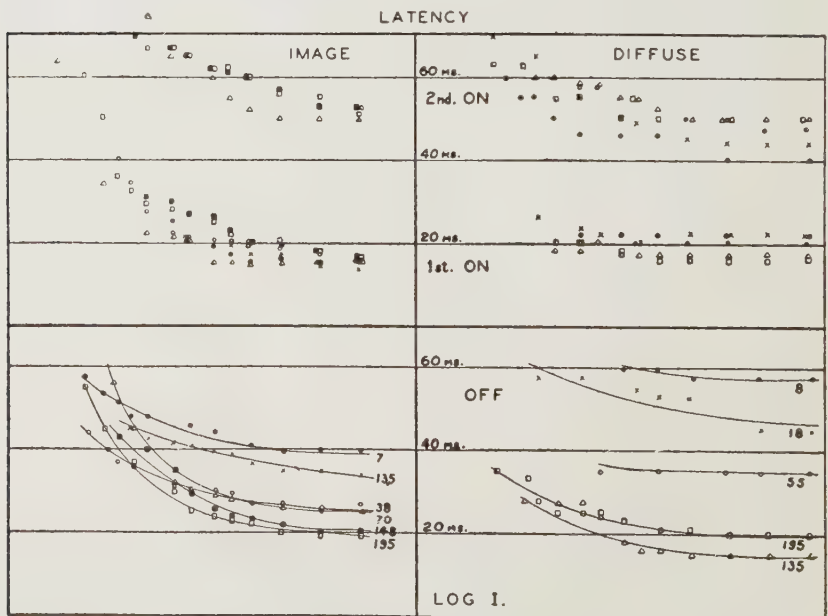


FIG. 2.

Latency of response vs. log. intensity for the three prominent waves of optic nerve. Left, 10 sq mm image on retina; right, diffusing screen at cornea, nearly whole retina illuminated. Range of intensity, 18,000 to 500 candles per sq ft. Stimulus duration in milliseconds at right.

these two responses. With decrease of either intensity or duration, the latencies of all responses increase. (Fig. 2.)

We infer from such records that the retinal activity arises in elements distal to the ganglion cell layer, and probably in the sense cells; and that the total *b* wave represents the summation of impulses which are individually briefer. Records obtained with and without a diffusing screen are so closely similar as to indicate that the responses observed following projection of a small bright image on the retina are chiefly due to stray light, illuminating the retina as a whole by internal dispersion from the image.

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Influence of Aldehydes on Transplanted Tumors.

CHRISTOPHER CARRUTHERS. (Introduced by E. V. Cowdry.)

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Strong obtained retrogressive changes and liquefaction of spontaneous tumors in mice^{1, 2} and in dogs³ by heptaldehyde. He also observed that the low boiling fraction of the oil of gaultheria was more effective than heptaldehyde alone,⁴ an effect probably due to the presence of naturally occurring antioxidants which prevent the auto-oxidation of heptaldehyde. Boyland and Mawson⁵ were able to induce some inhibition of both grafted and spontaneous tumors with citral, but heptaldehyde only inhibited the latter.

On the other hand, Baumann, Kline, and Rusch⁶ were unable to influence a spontaneous mammary adenocarcinoma, or tumors induced by ultraviolet light, or by benzpyrene by adding heptaldehyde to the diet of mice. Clarke⁷ found that heptaldehyde had no significant effect upon a transplanted spindle cell sarcoma in 14 rats. Orr and Strichland⁸ were likewise unable to affect spontaneous and trans-

¹ Strong, L. C., *Am. J. Cancer*, 1939, **35**, 401.

² Strong, L. C., *Science*, 1938, **87**, 144.

³ Strong, L. C., and Whitney, L. F., *Science*, 1938, **88**, 111.

⁴ Strong, L. C., *Yale J. Biol. and Med.*, 1938-39, **11**, 207.

⁵ Boyland, E., and Mawson, E. H., *Biochem. J.*, 1938, **32**, 1982.

⁶ Baumann, C. A., Kline, B. E., and Rusch, H. P., *PROC. SOC. EXP. BIOL. AND MED.*, 1938, **39**, 354.

⁷ Clark, W. G., *PROC. SOC. EXP. BIOL. AND MED.*, 1939, **40**, 562.

⁸ Orr, J. W., and Strichland, L. H., *Yorkshire Council of the British Empire Cancer Campaign*, 1938-39, 8.

planted tumors in mice, guinea pigs and in a dog by oral administration or injection of heptaldehyde.

Experiments were therefore undertaken to study the effect of aldehydes, especially heptaldehyde and some of its compounds, on transplanted carcinomas.

*Oral administration of pelargonic aldehyde and of heptaldehyde.** Emulsion from one tumor was injected at the same time into enough mice to serve for the experimental groups and for controls. The food was Purina Chow. As soon as tumors became palpable, the mice were divided into groups of comparable tumor diameters. One group received pelargonic aldehyde, one received heptaldehyde, while the other served as controls. Forty to fifty mg of the aldehyde were administered orally every day until the experiment was terminated. The product of the maximum length and width ($L \times W$), was used as a criterion for the growth rate of the tumors. The results of this experiment are indicated in Table I. The products of the tumor dimensions are recorded every 5 days and the increase in size can be observed as one reads the table from left to right. The first column shows the number of mice for the different groups with letters to indicate the type of treatment. The product of the tumor dimensions for any 5-day period for any of the 3 particular groups can be found by reading under the letter in question. Since Strong's outstanding results were with small tumors, in this and in the following experiments, treatment was begun when tumors were either not palpable, or were very small, in order to study the effect of aldehydes upon tumors before they became so large that any inhibitory effect might not be indicated. Hence, there are as many horizontal columns as there are tumors grouped according to initial tumor dimensions. There is no significant inhibition on the growth rate of the transplanted Marsch Buffalo Adenocarcinoma even when the aldehydes were administered to mice bearing small tumors.

To check the possibility that oral administration of heptaldehyde might have an effect on a transplantable tumor not manifested by changes in the growth rate, portions of a tumor were removed from a mouse which had received heptaldehyde (40-50 mg) daily for 27 days, and the tumor suspension was inoculated into 8 females. Since tumors developed in all the mice and grew rapidly, it is evident that the ability of the tumor to "take" was not lost.

To determine whether heptaldehyde given before transplantation would have any effect upon the subsequent growth of the Marsch Buffalo Adenocarcinoma, 20 mice were fed 40-50 mg of the alde-

* From Eastman, redistilled weekly and stored at 0-5°C.

TABLE I.
Effect of Oral Administration of Heptaldehyde and of Pelargonic Aldehyde on the Marsch Buffalo Adenocarcinoma.
Product of average tumor dimensions ($L \times W$) in cm at days after treatment was started.

[illegible]

TABLE II.
Effect of the Intraperitoneal Injection of Heptaldehyde on the A Carcinoma.

Sum of average tumor dimensions (L + W) in cm at days after treatment was started.																				
No. of mice			Days																	
			0			5			10			15			20			25		
			Initial tumor (L + W) cm																	
C	H	E	T.N.P.			T.P.			0-1.0			1-2.0			2-3.0					
0	4	0	C	H	E	C	H	E	C	H	E	C	H	E	C	H	E	C	H	E
6	10	4	1.7	1.1	1.7	2.8	1.8	2.9	3.3	2.7	3.8	4.7	3.9	4.8	4.8	4.8	5.8	4.8	4.8	5.8
2	1	0	2.2	1.8		3.4	2.8		3.9	3.8		5.3	4.4		5.8	5.0				
0	0	1			2.2			3.1			3.9			4.7						
0	1	0		3.7			4.3			5.3		6.1								6.6

C—Control; H—Heptaldehyde; P—Pelargonic Aldehyde; E—Ethyl Esters of Lard; T.N.P.—Tumors not Palpable; T.P.—Tumors Palpable.

hyde orally every other day for 19 days prior to inoculation of the tumor, and up to the time the experiment was terminated (25 days). Seven mice served as controls. Nineteen tumors occurred in the experimental group and in all of the controls, and the subsequent growth rate of the tumors in both groups was not significantly different.

Intraperitoneal injection of heptaldehyde. It has been observed that heptaldehyde is capable of causing resorption of mouse embryos, and is especially effective when dissolved in the ethyl esters of lard and injected intraperitoneally.⁹ On the assumption that the amount of aldehyde reaching the tumor after oral administration is less than after intraperitoneal injection, an attempt was made to inhibit the growth of transplanted tumors by intraperitoneal injection. Twenty-eight strain A mice were inoculated with an emulsion obtained from a spontaneous mammary carcinoma of the same strain, and as soon as tumors became palpable in some of the mice, heptaldehyde was injected intraperitoneally in graded doses because of its toxicity every day into 16 mice: For 3 days .03 cc of aldehyde in .06 cc ethyl esters of lard, for 2 days .045 cc of the aldehyde in .075 cc ethyl esters of lard, for 8 days .06 cc of the aldehyde in .06 cc ethyl esters of lard, for 13 days .09 cc of the aldehyde in .075 cc ethyl esters of lard.

Five mice were likewise treated with the ethyl esters of lard without aldehyde, while 8 served as untreated controls.

The sum of the maximum length and width ($L + W$) in cm was used as a criterion for the growth rate. Since the treatment was started as soon as a few tumors became palpable (in order to study the effect of heptaldehyde on the growth of tumors not palpable when the treatment was started), the initial tumor size for 3 groups varied. The results given in Table II show that heptaldehyde did not inhibit the growth rate of this carcinoma when the initial tumors were small (T.P.), and, also, that it was ineffective even when the tumors were not palpable (T.N.P.) when the injections were started. Similar results were obtained for the transplanted Marsch Buffalo Adenocarcinoma in 9 mice in which the tumors were either not palpable or were very small when the treatment was started.

Intraperitoneal injection of n-heptaldoxime. N-heptaldoxime (Eastman) was dissolved in the ethyl esters of lard (.05 cc contained 2 or 4 mg of the oxime) and its effect upon the 2 transplanted tumors was studied. As soon as tumors became palpable after transplantation, each mouse received 2 mg of the oxime per day for 2 days, then

⁹ Carruthers, C., PROC. SOC. EXP. BIOL. AND MED., 1939, **41**, 336.

TABLE III.
Effect of Intraperitoneal Injection of N-heptaldoxime on A Carcinoma and on Marsch Buffalo Adenocarcinoma.
Sum of average tumor dimensions (L + W) in cm at days after treatment was started.

No. of mice	Days																
	0	5			10			15			20			25			
	A Carcinoma.																
	Initial tumor (L + W) cm																
C	R	E	C	R	E	C	R	E	C	R	E	C	R	E	C	R	E
1	1	0	1.7	1.3	1.5	2.0	2.1	2.8	2.5	3.1	—	4.3	3.9	3.8	4.3	4.4	4.4
6	10	3	2.9	2.2	2.4	3.3	2.9	2.9	3.5	3.5	3.4	4.3	3.9	3.8	4.3	4.4	4.4
0	1	1	2.1-3	3.7	3.4	4.6	4.1	5.2	4.6	4.9	—	4.3	3.9	3.8	4.3	4.4	4.4
			Marsch Buffalo Adenocarcinoma.														
			T.P.	.6		1.3		2.6									
1			0-1.0	1.9	2.0	2.0	2.9	3.2	3.0	3.7	4.1	3.8	3.7	4.1	4.0	4.3	4.4
5	6	4															

TABLE IV.
Effect of the Bisulfite Addition Compound of Heptaldexide on A Carcinoma.

No. of mice		Days											
		0		5		10		15		20		25	
C	R ¹	Initial tumor (L + W) cm										C	R ¹
	3	T.N.P.											
	6	T.P.											
	2	0-1.0											
	1	1.1-2.0											
		C	R ¹	C	R ¹	C	R ¹	C	R ¹	C	R ¹	C	R ¹
			1.0		1.8		2.8		3.3		2.7		4.1
			1.8		2.8		3.4		3.9		3.5		4.7
		1.7		2.2		3.4		4.0		6.0		5.3	
			3.7		4.3							6.8	
												5.8	
												7.0	

C—Control; R—N-Heptaldoxime; E—Ethyl Esters of Lard; R¹—NaHSO₃—Addition Compound; T.N.P.—Tumors Not Palpable; T.P.—Tumors Palpable.

4 mg per day until the experiment was terminated. One group of mice served as controls, while another received injections of the ethyl esters of lard. Evidently (Table III), n-heptaldoxime had no significant effect upon these 2 transplanted tumors even when treatment was started when they were small (T.P. and [L + W] of O-2.0).

Subcutaneous injection of the bisulfite addition compound of heptaldehyde. Heptaldehyde was added to a saturated solution of NaHSO_3 , the addition compound was thoroughly washed with distilled water, and dried over CaCl_2 . The addition compound was made weekly and dissolved in distilled water prior to subcutaneous injection. In a preliminary experiment 5 mg of the addition compound were injected daily into 6 strain A mice bearing the A carcinoma, but no inhibitory effect was noticed, although the tumors were small when the treatment was started. Later 20 mg of the compound were injected daily into the same strain bearing the same carcinoma, but the results (Table IV) give little indication of any inhibition even when the initial tumor (L + W) is small (T.P.), or even when tumors could not be palpated when treatment was started (T.N.P.). Nine mice bearing the transplanted Marsch Buffalo Adenocarcinoma were also treated with 20 mg of the addition compound daily, with the same results. Mice bearing both of these tumors were also treated with an equivalent amount of NaHSO_3 , but without effect.

None of the above compounds induced noticeable liquefaction of the tumors. Subcutaneous injection of heptaldehyde resulted in necrosis which prevented this type of administration. Heptaldehyde ammonia had a similar effect and it was highly toxic via the intra-peritoneal route.

Strong's success with heptaldehyde was with spontaneous tumors. Practically no effect has been demonstrated on transplanted tumors. The reason for this difference is unknown. However, Cramer and Horning¹⁰ have shown that "brown degeneration" occurs in the adrenals, at least in several strains of mice which have a high incidence of spontaneous mammary carcinoma. Since spontaneous tumors arise in older females, the question arises as to the relative efficacy of heptaldehyde in young and in old mice bearing tumors, especially in the latter, because "brown degeneration" of the adrenals may be indicative of other endocrine changes, thus possibly allowing greater aldehyde activity. "Brown degeneration" has been observed in the adrenals of strain A mice in this laboratory, but was not a

¹⁰ Cramer, W., and Horning, E. S., *Lancet*, 1939, 192.

complicating factor as young mice were used as hosts for transplanted tumors. Bischoff and Long¹¹ have shown that sarcoma 180 grew to a greater size in young Marsch Buffalo mice than in older mice. This strain of mice also develops spontaneous mammary carcinoma.

On the other hand, Strong's results are obtained over a long period of time and in most of his experiments the aldehyde was mixed with the diet. This procedure might allow the proöxidant, heptaldehyde, by initiating autoöxidation or by the formation of peroxides, to induce dietary changes and thus influence tumors indirectly. The odor of heptaldehyde disappears quite rapidly when mixed with diets at room temperature.

Experiments are in progress to determine whether or not heptaldehyde will inhibit the growth of transplanted tumors in mice whose vitamin E stores have been depleted, since the tocopherols are known antioxidants¹² which may inhibit, at least partially, the action of the proöxidant, heptaldehyde.

Summary. Under the conditions of these experiments, the growth rates of the transplanted Marsch Buffalo Adenocarcinoma, of the transplanted A carcinoma were not significantly altered when: (1) heptaldehyde and n-heptaldoxime were dissolved in the ethyl esters of lard and injected intraperitoneally; (2) the bisulfite addition compound of heptaldehyde was injected subcutaneously. The oral administration of heptaldehyde and of pelargonic aldehyde was also without influence on the progress of the transplanted Marsch Buffalo Adenocarcinoma.

¹¹ Bischoff, F., and Long, M. L., *Am. J. Cancer*, 1936, **27**, 104.

¹² Oleott, H. S., and Emerson, O. H., *J. Am. Chem. Soc.*, 1937, **59**, 1008.

Change of the Age of Puberty in Albino Rats by Selective Mating.*

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During 3 decades, ending about 1922, the reported age of puberty in albino rats shifted downward approximately 20 to 30 days. This change has been ascribed in part to improved diets and husbandry, in part to the use of criteria based on estrus and copulation, rather than on fecundation, and in part to other still unrecognized causes. Among the latter, selective breeding has long been suspected of playing an influential rôle in view of the fact that animal breeders are not able to separate accurately the genetically late pubescent animals from those actually retarded because of illness, malnutrition, over crowding, or other adventitious circumstances contributing to what they consider undesirable breeding stock. A confusion of this kind would, in time, reduce the proportion of genetically late maturing animals in the breeding colony and thus tend to lower the mean ages of puberty in representative animals from the colony. To throw some light on the readiness with which selective breeding might affect mean age of pubescence, the present experiment was undertaken.

In starting the experiment, 25 pairs of albino rats, descendants of the inbred Slonaker colony and originating approximately 35 years ago from Wistar stock, 6 months of age and known to be fertile were mated and allowed to produce one litter each; from these the parent generation was selected. Although we realized that a still larger number of pairs was desirable in order to provide a goodly number of extreme cases, facilities at our disposal at that time prevented our exceeding the number specified above. Nothing was known concerning the ages of pubescence of these 25 pairs.

From the 25 litters 62 males and 82 females were reared. So far as possible living conditions for all of the litters were kept similar and a regular routine of feeding, handling, and examining the young was adopted so as to minimize variables in this sphere of influence. All of this generation and those which followed were fed the Steenbock mixture, supplemented with lettuce once a week. The ages of pubescence of the females were determined from vaginal

* This study was financially supported by a grant from the Committee for Research on Sex Problems, National Research Council.

smears; those of the males by direct tests for copulation. The mean age of first estrus for the 82 females was 51.1 days; the mean age at first copulation by the males was 58.3 days. The extremely early and extremely late cases were used as the parental generation.

The sire used from the parental generation representing the early strain copulated at the age of 44 days, and the 11 dams of the early strain were first in estrus at ages ranging from 35 to 43 days (mean, 40.0 days). For the late strain there were 2 sires, one copulating at the age of 70 and the other at the age of 76 days, and 10 dams with ages of first estrus ranging between 58 and 73 days (mean, 65.3 days). In the 6 succeeding generations the early pubescent animals selected for breeding never exceeded the puberal age of 49 days; the means for males and for females ranged between 37 and 43 days, with that of the males always slightly lower. The puberal age of males and females representing the late strain never fell below 55 days. For the late males the means ranged between 70 and 75 days and for females between 62 and 65 days.

The cumulative effects of selective breeding on age of puberty shown by the F_6 generation is given in Table I and is graphically

TABLE I.
Frequency Distributions of Ages of Puberty in Albino Rats of the 6th Selectively Bred Generation.

Ages	Early Males	Early Females	Late Males	Late Females
34-36	19	11	1	0
37-39	16	21	0	1
40-42	20	31	1	1
43-45	23	26	2	8
46-48	29	26	2	12
49-51	18	10	6	7
52-54	11	1	9	12
55-57	8	1	10	14
58-60	6	0	10	8
61-63	6	0	9	6
64-66	3	0	7	2
67-69	0	0	7	6
70-72	2	1	9	5
73-75	0	0	1	2
76-78	0	0	1	1
79-81	3	0	2	1
82-84	0	0	1	1
85-87	0	0	0	2
88-90	0	0	1	0
91-93	0	0	0	0
94-96	1	0	0	0
97-99	1	0	1	0
Total	166	128	80	89
Mean	47.56	42.98	61.18	56.87
σ	10.56	5.07	10.47	10.38

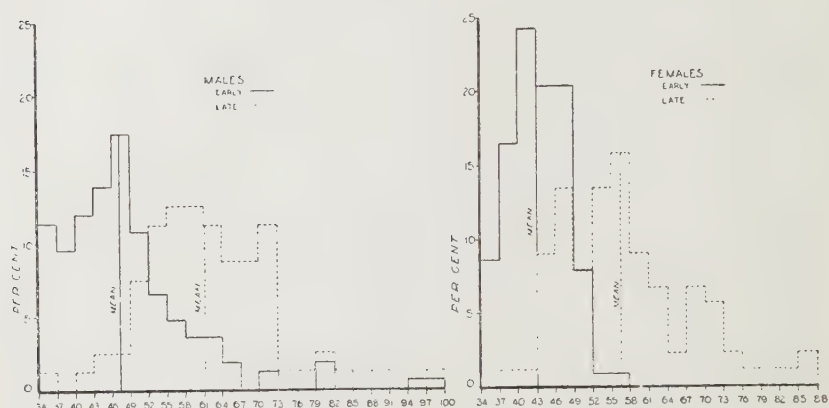


FIG. 1.

represented in Fig. 1. The mean ages of puberty for early and late males of the F_6 generation differ by 13.6 days; those for females by 13.9 days. For each sex the difference in means is statistically significant.

As the distributions clearly show, there is still a large amount of overlapping in the two strains. Further selective breeding should reduce this amount, but it is unlikely that it will eliminate it entirely. Two reasons for this may be given. Mild cases of illness and malnutrition tend to retard a few animals of the early strain in each generation and thus contribute to overlapping. Also the experimenter is unable to differentiate with the required exactitude those animals that are genetically late in maturing from those that are late because of both the genetic factor and adventitious factors that cause retardation. Thus he is not able to obtain in every instance the very best breeders to represent the late strain.

Summary. In this paper evidence is given which clearly indicates the possibility that lowering of the mean age of puberty in rat colonies during the past 30 years may have been due, in part, to selective breeding of early pubescent stock.

11351 P

Bacteriostatic Action of Various Wetting Agents upon Growth of Tubercle Bacilli *in vitro*.*

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Wetting agents in general possess the properties of penetrating and emulsifying surface films of fatty substances to a high degree, and are powerful surface tension reducers. For these reasons, it is of interest to observe their action on tubercle bacilli with their enveloping fatty capsule. The bactericidal action of several wetting agents on some organisms have been investigated, but little has been done in testing the effect of these compounds on the growth of tubercle bacilli.

The following compounds were tested for bacteriostatic action: Naconol NR,† an alkyl aryl sulfonate; Aerosol OT-100, an ester of a sulfonated bi-carboxylic acid; Aerosol OS, a sodium salt of an alkyl naphthalene sulfonic acid; and Zephiran, a mixture of high molecular alkyl-dimethyl-benzyl-ammonium chlorides. All the agents were approximately neutral in solution.

A non-virulent Novy strain of tubercle bacilli was grown on liquid synthetic asparagin media containing 6% glycerin, with pH 7.4. This produces a heavy surface growth in 2 to 3 days. Dilutions of the wetting agents were made in normal saline and added in equal amounts to tubes containing 4.5 cc of media. A large loopful of surface growth of a 3-day culture of tubercle bacilli was inoculated on a cork float in each tube. The tubes were then slanted and incubated at 37°C. Readings of the amount of growth were made at 2-day intervals.

The results are shown in Table I. The most effective wetting agent is Zephiran, which produced complete inhibition of growth in dilution of 1:80,000, and retardation of growth in dilutions as high as 1:400,000. Naconol NR and Aerosol OT-100 prevented growth in dilution of 1:5,000, and retarded growth in dilution of 1:40,000. Aerosol OS produced the lowest bacteriostatic action,

* This investigation was aided by a grant from the San Francisco Tuberculosis Association.

† Naconol, a product of the National and Chem. Co.; Aerosol, a product of the Amer. Cyanamid & Chem. Corp.; Zephiran, a product of the Alba Pharmaceutical Co.

TABLE I.
Action of Four Wetting Agents on Growth of Tubercle Bacilli *in vitro*.

Dilution	Growth											
	Zephiran			Nacconol			Aerosol OT-100			Aerosol OS		
	2 days	4 days	6 days	2 days	4 days	6 days	2 days	4 days	6 days	2 days	4 days	6 days
1:1,000	—	—	—	—	—	—	—	—	—	1+	4+	—
1:5,000	—	—	—	—	—	—	—	—	—	2+	4+	—
1:10,000	—	—	—	—	—	—	—	—	—	2+	4+	—
1:15,000	—	—	—	—	—	—	—	—	—	2+	4+	—
1:20,000	—	—	—	—	—	—	—	—	—	2+	4+	—
1:40,000	—	—	—	—	—	—	—	—	—	2+	4+	—
1:50,000	—	—	—	—	—	—	—	—	—	2+	4+	—
1:60,000	—	—	—	—	—	—	—	—	—	2+	4+	—
1:70,000	—	—	—	—	—	—	—	—	—	2+	4+	—
1:80,000	—	—	—	—	—	—	—	—	—	2+	4+	—
1:90,000	—	—	—	—	—	—	—	—	—	2+	4+	—
1:100,000	1+	1+	3+	1+	1+	2+	1+	1+	2+	1+	4+	—
1:125,000	1+	1+	3+	1+	1+	2+	1+	1+	2+	1+	4+	—
1:150,000	1+	1+	3+	1+	1+	2+	1+	1+	2+	1+	4+	—
1:200,000	1+	1+	3+	1+	1+	2+	1+	1+	2+	1+	4+	—
1:250,000	2+	2+	4+	2+	2+	4+	2+	2+	4+	2+	4+	—
1:300,000	2+	2+	4+	2+	2+	4+	2+	2+	4+	2+	4+	—
1:350,000	2+	2+	4+	2+	2+	4+	2+	2+	4+	2+	4+	—
1:400,000	2+	2+	4+	2+	2+	4+	2+	2+	4+	2+	4+	—
1:450,000	3+	3+	4+	3+	3+	4+	3+	3+	4+	3+	4+	—

Controls. 3+ in 2 days; 4+ in 4 days.

permitting growth in dilution of 1:1,000, and retarding growth slightly in dilution of 1:10,000. The above results are not strictly comparable, as the degree of chemical purity of each of the compounds is unknown.

Although the bacteriostatic action of Zephiran against tubercle bacilli is high, its bactericidal action is low. To 4.5 cc of a 1:1000 dilution of Zephiran was added $\frac{1}{2}$ cc of an undiluted 3-day growth of tubercle bacilli, which previously had been well shaken to break the surface film into small particles. This mixture was incubated at 37°C for $\frac{1}{2}$ hour, then centrifugalized, washed twice with normal saline, and the sediment planted on Lowenstein's egg media. The growth was positive.

The superior bacteriostatic action of Zephiran over that of the other wetting agents is not due entirely to its ability to reduce surface tension or to superior wetting qualities, as these qualities are approximately the same in all the compounds tested. Its superior action is probably inherent in its molecular structure.

11352 P

Functional Transplants of Epithelial Hypophysis in Three Species of Amblystoma.

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Previously it has been shown^{1, 2} that transplantation of the primordium of the epithelial hypophysis, independent of brain and foregut, may be followed by differentiation and function of the transplant in 2 species of frogs, *Rana sylvatica* and *R. pipiens*. Although numerous transplants were attempted with embryos of *Amblystoma punctatum* no unequivocally successful cases were obtained. This seemed to confirm the conclusions previously reached by Blount,³ who held that the epithelial portion of the hypophysis is dependent upon an association with the neural lobe for its differentiation and functioning.

More recently Blount⁴ has presented evidence to show that hypo-

¹ Atwell, W. J., *Proc. Soc. Exp. Biol. and Med.*, 1935, **33**, 224.

² Atwell, W. J., *Anat. Rec.*, 1937, **68**, 431.

³ Blount, R. F., *J. Exp. Zool.*, 1932, **63**, 113.

⁴ Blount, R. F., *Anat. Rec.*, 1939, **73**, Sup. 1, 7.

physeal transplants in *A. punctatum* may produce thyreotropic hormone. Regarding the pars intermedia, however, he states that this lobe "does seem dependent upon the pituitary floor of the diencephalon for its development." Burch,⁵ by operations performed on *Hyla regilla* in the gastrula stage, was able to suppress the differentiation of the pars intermedia and thus to produce silvery animals. Eakin⁶ undertook experiments upon *Triturus torosus*, to confirm Burch's findings. At a stage corresponding to Stage 36 in Harrison's series gelatin was injected into the fore-gut. Following this procedure a single case of 'albinism' was found. This, together with the histological picture, was taken to indicate that the differentiation of the epithelial hypophysis is dependent upon the inducing power of the infundibulum.

The present experiments were performed upon embryos of *A. jeffersonianum*, *A. tigrinum*, and of the white axolotl (partial-albino strain of *A. mexicanum*). Operations were done at the tail-bud stage, corresponding approximately to Harrison's stages 30-32 for *A. punctatum*. Using care not to remove any of the brain floor the solid primordium of the epithelial hypophysis with some of the associated ectoderm was transplanted in the same animal to a position between the right otocyst and the hind brain. A small incision through the epidermis permitted the insertion of the transplant. The point of entrance was dorsal and rostral to the otocyst. Animals were reared in the laboratory for from 2 to 4 months. Completely hypophysectomized animals without transplants and normal, unoperated salamanders served as controls.

For a transplantation experiment to be considered successful it was required that study of the animal and of the serial sections show: (1) entire absence of the epithelial hypophysis from the orthotopic position, (2) characteristic hypophyseal tissue in the region into which the transplant had been placed, and (3) some evidence of function of the transplant as described below. Forty such 'successful' cases are included in this report, 35 being of *A. jeffersonianum*, 3 of *A. tigrinum*, and 2 of the white axolotl. These 40 cases are from 72 operated animals studied at autopsy.

Most of the transplants were contained in the cartilage surrounding the internal ear. A few were dorsal, ventral, rostral or medial to the otic capsule. Only 2 were contained in the cranial cavity. Histologically the transplants showed the structure of the pars intermedia and/or of the pars anterior. In no case could a neural

⁵ Burch, A. B., PROC. SOC. EXP. BIOL. AND MED., 1938, **38**, 608.

⁶ Eakin, R. M., *Growth*, 1939, **3**, 373.

lobe or a distinct pars tuberalis be recognized. Some of the transplants consisted in part of epithelial cysts or vesicles, the derivation of which is not always clear. Some of them, however, exhibited the structure of the epidermis. A few may have been derived from cells inadvertently removed from the brain floor and included with the transplant.

The successfully transplanted cases gave evidence of function of the pars intermedia by being dark in color in striking contrast to the silvery condition of hypophysectomized animals without transplants. The dark color was observed to be due to dispersion of pigment in both dermal and epidermal melanophores. Several animals with transplants exhibited a more intense pigmentation than normal, unoperated controls.

In a number of cases the living animals showed a spot of still darker coloration, 3-4 mm in diameter, in the dorsal skin, caudal to the right eye. In a few instances this dark spot was located in the roof of the mouth or pharynx ventral to the internal ear. In each of the above cases study of serial sections revealed that the transplant was located in relation to the center of the dark area, being either subepidermal or submucosal. This was taken to indicate that in certain cases at least the transplant may exert a local as well as a systemic effect on pigment cells.

The function of the pars anterior of the transplanted hypophysis was evidenced by the normal size of the thyroid glands as contrasted with the atrophic condition found in hypophysectomized animals without transplants. None of the animals had completed metamorphosis although several had made noticeable progress in that direction.

Summary. Autoplastic, heterotopic transplants of the primordium of the epithelial hypophysis have been made in three species of *Amblystoma*. These transplants differentiated without contact with the brain, and gave evidence of chromatophorotropic and thyreotropic activity.

Treatment of *Bartonella muris* Infections with Sulfanilamide.

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Splenectomy in rats is often followed by anorexia, anemia, hematuria, emaciation, and death. The cause of these severe symptoms is known to be a parasitic infestation commonly spoken of as *Bartonella muris* infection.^{1, 2, 3} Although the treatment of this condition is unsatisfactory, certain substances are beneficial, such as, arsenicals,⁴ copper and iron,⁵ and extracts of spleen.⁵

The beneficial effect of sulfanilamide and related compounds in distemper has been shown in dogs by Marcus and Necheles,⁶ and in ferrets by Dochez and Slanetz.⁷ Negative results have been noted in canine distemper⁸ and in rats infected with *Trichinella spiralis*.⁹ The wide use of sulfanilamide in these, and in other blood infections, led to the present experiments with *Bartonella muris*.

The rats used in these experiments were nearly full grown albino males. They were kept in a room having a constant temperature of 26°C with a variation of 2°C. Their diet consisted of Purina dog chow and water. Splenectomy, performed under ether anesthesia, was done through a shaved area of the skin, sterilized with alcohol. The same day, blood from a splenectomized rat suffering from an acute infection of *Bartonella muris* was injected intraabdominally. Five cc of blood from the donor were mixed with 18 cc of 0.9% NaCl and 2 cc of a 25% solution of sodium citrate, making a total of 25 cc. Each recipient received 5 cc of this mixture. A 1% suspension of sulfanilamide was given intraabdominally twice daily for 5 days in 5 cc amounts or a total daily injected dose of 100 mg. Also, about 20 mg daily were eaten with ground wheat. This made a total

¹ Lauda, E., *Virchows Arch. path. Anat.*, 1925, **258**, 529.

² Ford, W. W., and Eliot, C. P., *J. Exp. Med.*, 1928, **48**, 475.

³ Emery, F. E., *et al.*, *Endocrinology*, 1940, **26**, 167.

⁴ Mayer, M., *et al.*, *Klin. Wschr.*, 1926, **5**, 559.

⁵ Perla, D., and Marmorston-Gottesman, J., *J. Exp. Med.*, 1932, **56**, 777, 783.

⁶ Marcus, P. M., and Necheles, H., *PROC. SOC. EXP. BIOL. AND MED.*, 1938, **38**, 385.

⁷ Dochez, A. R., and Slanetz, C. A., *Science*, 1938, **87**, 142.

⁸ Dickerson, V. C., and Whitney, L. F., *PROC. SOC. EXP. BIOL. AND MED.*, 1938, **38**, 263.

⁹ McCoy, O. R., *PROC. SOC. EXP. BIOL. AND MED.*, 1938, **38**, 461.

daily intake of approximately 120 mg and equal to at least 500 mg per kilo of body weight.

The sulfanilamide had no apparent effect on the *Bartonella muris*. All 15 of the treated rats gave positive blood smears, taken on the third to fifth day after being infected, and 10 died within 12 days. These were practically the same results as obtained in 15 untreated controls where 14 gave positive blood smears and 8 died. The effectiveness of sulfanilamide was further tested by adding 1% to the solution of physiological saline and sodium citrate just described for Group A. The donor blood was added to this 1% mixture and allowed to stand 10 minutes, then injected intraabdominally into the splenectomized recipient rats. The results again failed to show any beneficial effect from sulfanilamide; 13 of the 15 rats in this group showed positive blood smears and all were dead within 12 days. The toxicity of the sulfanilamide may have contributed to the high mortality in this group as compared to the controls. In all groups the *Bartonella muris* bodies disappeared rapidly from the blood and were usually not found after the fifth day, even though the symptoms were so severe that the rats continued in an emaciated condition for several more days. After the twelfth day, few died.^{2, 3}

Summary. Splenectomized rats infected with *Bartonella muris* were treated with sulfanilamide in doses of 500 mg per kilo of body weight. A study of 30 rats thus treated showed that the treatment had no detectable effect on *Bartonella muris*. The toxicity of the sulfanilamide seemed to be a factor contributing to the mortality.

11354

Effect of Thymectomy at Birth on Spermatogenesis in the Albino Rat.*

JAMES C. PLAGGE. (Introduced by Carl R. Moore.)

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Shay, *et al.*,¹ reported severe retardation of spermatogenesis following roentgen destruction of the thymus during the first few days of the rat's life. It would appear that if X-ray treatment of the

* This investigation has been aided by a grant from the Dr. Wallace C. and Clara A. Abbott Memorial Fund of The University of Chicago.

¹ Shay, Harry, Gershon-Cohen, Jacob, Fels, Samuel S., Meranze, David R., and Meranze, Theodore, *J. Am. Med. Assn.*, 1939, **112**, 290.

thymus of newborn rats exerts such an influence on the testes, then complete surgical removal of the thymus at the same age would achieve similar results. This paper reports results of thymectomy in newborn albino rats with special reference to the progress of spermatogenesis.

Methods and Materials. The histological criterion for spermatogenesis used was that reported by Moore² in which the "sperm-head stage" appears between 33 to 35 days of age.

Forty-six male albino rats, representing 11 litters, were used. In all cases the litter was employed as the unit. The testes of thymectomized rats were compared with those of unoperated or sham-operated littermate controls. The thymus was removed before the animal was 24 hours old. Operations were performed under ether anesthesia or after chilling the animal. A mid-ventral section of the anterior end of the sternum was made back to the 4th intercostal space. Only those cases have been considered in which the thymus was removed *in toto* without surgical complications; in which no accessory cervical thymic tissue was seen at the time of the operation; and in which histological examination of suspected thymus rests, recovered at autopsy and sectioned serially, proved not to be thymus tissue. Both thymectomized animals and their littermate controls were weighed at 5-day intervals and were sacrificed at 33, 37, and 38 days.

Testes and seminal vesicles were weighed fresh and fixed in Bouin's solution. Complete cross sections of testes were cut at 7 micra and stained with Ehrlich's hematoxylin. Fifty seminiferous tubules were inspected in each of 5 different sections. The percentage of tubules containing sperm heads in the 250 tubules thus examined per animal was recorded as an index of the amount of spermatogenesis that had taken place.

Effect on testis. Table I presents data recorded for 46 rats, 25 of which were completely thymectomized at birth. The results of sperm head counts in exactly 250 seminiferous tubules per animal are expressed in percentage. It will be seen that all 9 thymectomized rats from 4 litters sacrificed at 33 days of age failed to show any sperm heads in the testes. In 5 of the 7 littermate controls, autopsied at the same time, sperm heads were completely missing. In 2 of the controls, 0.8% of the tubules contained sperm heads.

Out of 14 thymectomized rats sacrificed at 37 days of age, 12 showed sperm heads in 10% to 42% of the tubules examined, and 2 (Nos. 118 and 119) failed to exhibit sperm heads. Two unop-

² Moore, Carl, R., *Am. J. Anat.*, 1936, **59**, 63.

SPERMATOGENESIS IN THYMECTOMIZED ALBINO RATS 59

TABLE I.
Spermatogenesis in Rats Thymectomized at Birth.

Litter No.	Rat No.	Age in days	% of seminiferous tubules with sperm heads*		
			Thymec- tomized	Controls	
				Not operated	Operated
52	85	33	0		
	86	33	0		
	87	33		0	
	88	33		0	
53	89	33	0		
	90	33	0		
	91	33	0		
	92	33			0
	93	33		0.8	
59	108	33	0		
	109	33	0		
	110	33			0
	111	33			0.8
58	101	33	0		
	103	33	0		
	106	33		0	
	102	37	10		
	104	37	23		
	105	37	22		
	107	37		20	
49	78	37	23		
	79	37		37	
	80	37		31	
50	81	37	42		
	82	37	18		
	83	37		31	
	84	37		24	
54	94	37	21		
	95	37	25		
	96	37			26
57	97	37	30		
	98	37		0	
	99	37		5	
60	113	37	30		
	114	37	30		
	115	37		31	
	116	37		34	
61	117	37	11		
	118	37	0		
	119	37	0		
	120	37		0	
	121	37		0	
48	74	38	33		
	75	38	24		
	76	38		29	
	77	38		31	

*250 seminiferous tubules examined in each animal.

erated littermate controls of the latter (Nos. 120 and 121) also revealed no sperm heads. One other control (No. 98) failed to show sperm heads on this day, whereas 9 had sperm heads in from 5% to 37% of the tubules observed.

In one litter of 4 males sacrificed at 38 days of age, both the 2 operated and the 2 control animals showed sperm heads in 24% to 33% of the tubules.

Neither absolute nor relative fresh testicular weights were influenced by thymectomy. This is an interesting point in view of the fact that gonadectomy of newborn male or female rats causes a definite hypertrophy of the thymus.³

These results indicate that thymectomy of the newborn male rat neither accelerates nor retards the process of spermatogenesis as judged by the first appearance of sperm heads in the testis.

Effect on seminal vesicle. Gross and histological examinations were made of the seminal vesicles of thymectomized rats and their respective littermate controls. The histology of the seminal vesicle has been described as a reliable indicator of male hormone secretion.⁴ At 33 days of age both thymectomized and control rats showed granular cytoplasm in the epithelium of the seminal vesicle. At 37 and 38 days distinct secretion granules were found in the vesicles of operated and control animals. Furthermore, the absolute and relative fresh weights of the seminal vesicles were practically the same for both groups.

Individual body weights, recorded at 5-day intervals, gave no indication that thymectomy in rats at birth, if unaccompanied by surgical complications, has any influence upon general body growth up to 37 days of age.

Summary. Twenty-five male albino rats from 11 litters were thymectomized at birth. These were sacrificed at the ages of 33, 37, or 38 days. Testes and seminal vesicles were compared with 21 unoperated or sham-operated littermate controls. The results indicate that complete thymectomy of newborn rats neither hastens nor retards spermatogenesis, hormone output or growth of the testis.

³ Plagge, James C., unpublished.

⁴ Moore, Carl R., Hughes, Winifred, and Gallagher, T. F., *Am. J. Anat.*, 1930, 45, 109.

Failure of Ovarian Hormones to Cause Mating Reactions in Spayed Guinea Pigs with Hypothalamic Lesions.*

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It has been reported that female cats with small lesions in the hypothalamus in such a position as to interrupt the supraoptico-hypophysial tract were not observed to come into heat and were never bred in the laboratory.¹ It has more recently been found that the production of small lesions in a comparable part of the hypothalamus of the female guinea pig is followed by a complete lack of the mating response and in some cases also by disturbances in the ovarian cycle.² Although the disturbances in the sexual cycles may be attributable to a secondary disruption of hypophysial function, the majority of the animals showed regular sexual cycles which were normal so far as the physical changes in ovaries, uteri and vaginae were concerned, suggesting that their hypophyses were functioning normally. The present investigation was undertaken in order to determine whether the lack of the mating response in guinea pigs following hypothalamic lesions of the type described is due to an hormonal insufficiency or to the destruction of neural elements indispensable to the estrous or mating reflex.

Marrian and Parkes³ have shown that vaginal estrus may be brought about by an amount of estrogen which is insufficient to induce uterine changes or copulatory behavior. On the other hand, Dempsey and Rioch⁴ were unable to induce behavioral estrus in a guinea pig following removal of the brain rostral to a plane extending between the anterior limits of the superior colliculus and the posterior edge of the mammillary bodies, although the reflex arc remained intact when the ventral limit of the section was in front of the mammillary bodies. On the basis of this evidence they have postulated a sexual center located in the ventral hypothalamus at the

* Aided by a grant from the Committee for Research in Problems of Sex of the National Research Council.

¹ Fisher, C., Magoun, H. W., and Ranson, S. W., *Am. J. Obstet. and Gynec.*, 1938, **36**, 1.

² Dey, F. L., Fisher, C., Berry, C. M., and Ranson, S. W., *Am. J. Physiol.*, 1940, **129**, 39.

³ Marrian, G. F., and Parkes, A. S., *J. Physiol.*, 1930, **69**, 372.

⁴ Dempsey, E. W., and Rioch, D. M., *J. Neurophysiol.*, 1939, **2**, 9.

level of the mammillary bodies. Bard,⁵ however, has reported that estrous responses may be elicited in cats following massive lesions in the posterior hypothalamus which destroy all known descending paths from that part of the brain, and believes that the integration of the reflex is a mesencephalic function.

A series of 27 young, adult female guinea pigs, weighing between 400 and 600 g, were ovariectomized. Following a recovery period they were each brought into full behavioral estrus several times by the subcutaneous injection of 12.5-15.0 IU of estrogen† on hours 0, 24, 48, and 60, followed by 0.2 IU of progesterone‡ on hour 72, after the method described by Collins, Boling, Dempsey and Young.⁶ After the constancy of the response to ovarian hormones had been established in each animal, lesions were placed in the hypothalamus at the level of the posterior border of the optic chiasma with the aid of a Horsley-Clarke instrument bearing a unipolar electrode. Three lesions were placed in each animal, one in the midline and one on each side of the midline at a distance of one millimeter, by passing a direct current of 3.0 ma for 30 seconds. In 22 animals the lesions were placed 1 mm above the ventral surface of the brain, and in 5 animals the lesions were placed 6 mm above the ventral surface. Five of the animals with the low lesions failed to survive the operation. Gross inspection of the brains from these animals indicates that the lesion occurs just posterior to the optic chiasma. The remaining 22 animals recovered completely, grew normally, and remained in excellent condition for the duration of the experimental period. Aside from the diabetes insipidus which developed in some animals, and a transitory period of depression which lasted for approximately 12 hours after the operation, there were no criteria by which the operated animals could be differentiated from normal anestrous female guinea pigs.

At least 2 attempts have been made to induce estrus in 17 of these animals with lesions near the ventral surface of the brain, using the dose of ovarian hormones which was sufficient to alter the behavior of the animals before the lesion. None of the animals so treated showed either proestrous or estrous behavior. It was impossible to elicit the estrous reflex by manual stimulation of the vulva or the lumbo-sacral region of the back, and none of these animals would

⁵ Bard, P., *Res. Publ. Assn. Res. Nerv. Ment. Dis.*, 1940, **20**, 551.

† Theelin, through the courtesy of Dr. Oliver Kamm, Parke, Davis and Co.

‡ Proluton, through the courtesy of Dr. Erwin Schwenk, Schering Corp.

⁶ Collins, V. J., Boling, J. L., Dempsey, E. W., and Young, W. C., *Endocrinology*, 1938, **23**, 188.

accept the male. All gave good avoiding responses to such stimulation, after the manner of a normal anestrous female. In subsequent trials, 8 of the animals were injected with double the usual dose of hormones and failed to show estrus, while 4 of the animals were injected with quadruple the usual dose of hormones and also failed to come into heat.

That the failure of the ovarian hormones to induce estrus in these animals is not due to a non-specific effect of destruction in the central nervous system is shown by the experiments of Bard,⁷ Bard and Rioch,⁸ Brooks,⁹ Dempsey,¹⁰ and Davis.¹¹ In addition, the 5 animals which have had lesions placed 6 mm instead of 1 mm above the ventral surface of the brain have been brought into heat with the same dose of hormone which induced estrus before the lesion was made.

These results differ from those of Dempsey and Rioch⁴ and Bard.⁵ Dempsey and Rioch's localization of the sexual center is based primarily upon the results of acute experiments on one guinea pig and one cat. In their chronic experiments failure to induce estrus following the removal of the anterior hypothalamic region is attributed to the debilitating effect of the operation on the animal. In their acute experiments successive transections were made in the same animal at various levels of the brain stem either with a blunt spatula or with a small sucker. In such experiments the accuracy of the localization of a "center" depends entirely upon the accuracy with which the location of the destruction to the central nervous system can be determined. We believe it may be significant that out of the 5 cats reported upon by Bard, the lesion in the one animal which failed to come into heat extended farther forward than in the other 4 animals. Although the main body of the lesion in the cats involved all known descending tracts from the hypothalamus, the possibility of the conduction of descending impulses by other paths has not yet been ruled out.

Summary. Following appropriately placed lesions at the level of the posterior border of the optic chiasma, ovariectomized guinea pigs failed to respond to previously effective dosages of estrogen and progesterone. The results reported here indicate that the failure of these animals to show estrous behavior is not due to a lack of ovarian hor-

⁷ Bard, P., *Am. J. Physiol.*, 1936, **116**, 4.

⁸ Bard, P., and Rioch, D. M., *Bull. Johns Hopkins Hosp.*, 1937, **60**, 73.

⁹ Brooks, C. M., *Am. J. Physiol.*, 1937, **120**, 544.

¹⁰ Dempsey, E. W., *Am. J. Physiol.*, 1939, **126**, 758.

¹¹ Davis, C. D., *Am. J. Physiol.*, 1939, **127**, 374.

mones. It is possible that the lack of response to the hormones is a result of the destruction of a portion of the central nervous system which is indispensable to the integration of a complex behavior pattern. If further control experiments prove this to be the case, then the possibility must be considered that the integrating mechanism involved is located in the midventral portion of the anterior hypothalamus instead of the region of the mammillary bodies or the mesencephalic tegmentum.

11356

Selective Localization of Evans Blue (T1824) in Subplacental Portions of Entoderm in the Rat.*

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The dye, Evans blue (T1824), a non-toxic, azo-compound, isomer of trypan-blue, has been observed following intravenous injection to localize selectively in and about malignant neoplasms in animals¹ and in man² but does not localize selectively in and about benign tumors in man or animals.² In and about the malignant neoplasms it accumulates in the macrophages and fibroblasts of the stroma. It does not penetrate into the neoplastic cells themselves, whether they be carcinoma or sarcoma.

During the course of experiments to observe its localization in tumor-bearing rats a pregnant animal (about mid-term) was inadvertently employed. At necropsy 24 hours after intravenous injection of 4 mg of the dye dissolved in 1 cc distilled water, it was noted that in addition to the sarcoma there was marked selective concentration of the dye in that portion of the visceral entoderm subjacent to the disc-shaped placenta. The remainder of the visceral entoderm did not appear grossly to have localized the dye (Fig. 1). The uterine musculature appeared tinged lightly blue as did the placenta; the embryo, and the amniotic fluid contained no dye grossly visible.

To confirm these observations 7 pregnant female white rats were

* This work was carried out under a grant from the Cancer Research Institute of the Chicago Woman's Club, Chicago, Illinois.

¹ Duran-Reynals, F., *Am. J. Cancer*, 1939, **35**, 98.

² Brunschwig, A., and Clarke, T. H., *Am. J. Path.*, in press.

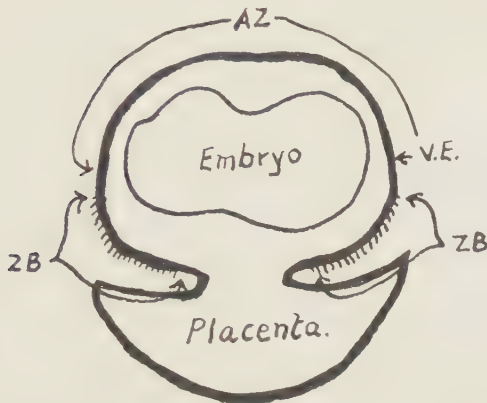


FIG. 1.

Diagram of cross-section of rat embryo in latter part of gestation. VE, visceral entoderm. ZB, subplacental portion of visceral entoderm with villous-like processes. These processes are not visible macroscopically. Selective localization of Evans blue was noted in this zone. AZ, anti-placental portion of visceral entoderm; cells are relatively lower in this region and no localization of the dye was noted here.

injected with similar quantities of dye, some at mid-term, and some just prior to the expected date of delivery. The animals were killed and at necropsy, 24 hours after injection, the concentration of the dye was observed in each instance as described above. Six pregnant females were injected with 4 mg of trypan blue. In 3 the localization occurred in the subplacental portion of entoderm as was found for Evans blue; in one the localization was not intense in this area, and in 2 it did not occur at all. Two pregnant females received intravenous injections of .5 cc India ink (diluted with water 1 to 3) and the latter localized definitely within the placenta as well as in the liver, spleen, bone marrow and some lymph nodes. The entire entoderm remained free from India ink.

Microscopic study of sections of the excised entoderms, portions of which had concentrated the Evans blue, and which were rapidly fixed, dehydrated and embedded to prevent loss of dye by diffusion, showed the dye within the columnar entoderm cells themselves as small aggregates of blue granules. The subplacental portions of the visceral entoderm are composed of tall columnar cells thrown into villus-like processes. The cells of the visceral entoderm became progressively flatter away from the placenta until over the area opposite the placenta where no dye was concentrated they are very low, flattened cells.

Discussion and Summary. The above described selective localization of Evans blue is of special interest since this localization in epithelial cells is in contrast to that observed when the dye concentrates selectively in and about carcinomas or sarcomas which localiza-

tion is in mesoblastic cells of the stroma—*i. e.*, fibroblasts and macrophages. Such selective concentration might denote a special physiologic property of a portion of the visceral entoderm in the type of placentation represented in the rat.

Goldmann³ in his studies on intravital staining showed that trypan blue was concentrated in all of the entoderm in mice. However, storage of this dye was also observed by him in all of the reticulo-endothelial system, of the mother, because of the relative enormous doses of the dye administered. Such very large doses precluded the demonstration of selective affinity for the dye on the part of certain tissues, as for example, the subplacental visceral entoderm. In his excellent monograph entitled "The Localization of Disease" Burrows⁴ illustrates in colors the exposed viscera of a pregnant rat injected with isamine blue. The uterine horns have not been opened and the dye appears localized in and about the placenta. The illustration was published to indicate specific concentration of the dye in the placenta. From our own studies in which the gross appearance of the unopened pregnant uterus was similar to that depicted by Burrows, we would raise the question of whether the dye he used was also concentrated not in the placenta primarily, but rather in the subjacent visceral entoderm as occurred with Evans blue in the observations reported above.

11357 P

Use of Orally Administered Desiccated Thyroid in Production of Traumatic Shock.

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In an attempt to study the effect of adrenal cortical extracts on traumatic shock, we observed that normal anesthetized dogs, following repeated trauma to the limbs, testicles and gut, did not go into shock at least within 8 hours. We discussed this problem with Dr. Ivy,¹ who had produced shock in dogs by trauma, and he suggested that a possible reason for his ability to induce shock in dogs might

³ Goldmann, E., *Beitr. zur Klin. Chirurg.*, 1909, **64**, 192.

⁴ Burrows, H., *Localization of Disease*, Wm. Wood & Co., London, 1932.

¹ Ivy, A. C., *Am. J. Physiol.*, 1920, **51**, 197.

have been due to the fact that most of the dogs he worked on had hyperplastic thyroid glands.

Following this information we performed the following experiments:

1. Thirty apparently normal dogs were anesthetized with ether, and blood pressure recorded by connecting the carotid artery with a mercury manometer. The limbs and testicles of these dogs were traumatized by 100 blows with a wooden mallet. We also manipulated the gut from $\frac{1}{2}$ to 1 hour.

2. In another series of experiments 18 dogs were fed .4 g of desiccated thyroid tissue per kg of body weight per day for 1 week, then were anesthetized, and treated as in the first series, blood pressure recorded, and their intestines manipulated from 15 to 20 minutes. The average results of both series of experiments are recorded on the graph (Fig. 1).

The graph shows that normal dogs after severe trauma, did not go into shock, while experimental hyperthyroid dogs, with much less trauma died of shock within $3\frac{1}{4}$ hours. The dogs which were fed thyroid were in apparently good condition. Although we did



FIG. 1.

Upper line shows average blood pressure of 30 normal dogs. Lower line shows average blood pressure of 18 hyperthyroid dogs. The arrow indicates the blood pressure 15 minutes after trauma.

not measure the B.M.R.'s of these dogs, studies on other dogs showed that this amount of thyroid usually increases the B.M.R. from 15 to 20%.

Unbeknown to us, Hepler and Simonds² had reported that dogs which were fed thyroid showed a greater drop in blood pressure than did normal dogs, when the hepatic veins were occluded for short intervals.

Further studies are being carried on to determine what changes occur that make it possible to produce traumatic shock in experimental hyperthyroid dogs.

Summary. The authors found that prolonged manipulation of the intestines of normal anesthetized dogs, does not produce shock. However, when dogs are fed .4 g of desiccated thyroid per kg of body weight per day for 1 week, these animals on manipulation of the intestines from 15 to 20 minutes, go into shock quite readily.

We wish to thank Dr. Carlson for his interest and advice during the course of these experiments.

11358 P

Accessory Growth Factor Requirements of Some Members of the Pasteurella Group.

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Most members of the Pasteurella group of bacteria develop satisfactorily in meat infusion-peptone media but fail to grow in simpler media made from hydrolyzed purified protein or in synthetic media. The substances in infusions of meat, other tissues or yeast which are needed for growth have not been previously identified. Accordingly we wish to report preliminary results of a study of the accessory growth factor requirements of some members of this group of organisms in which it will be shown that nicotinamide, pantothenic acid and, in some cases, the butyl factor for Clostridia are needed for prompt development.*

Seventeen typical *Pasteurella* strains were used. These were stock

² Hepler, Opal E., and Simonds, J. P., *Arch. Path.*, 1938, **25**, 149.

* We are indebted to Dr. E. E. Snell and Dr. R. J. Williams for the samples of pantothenic acid used in these experiments and to Dr. W. H. Peterson for the butyl factor.

laboratory cultures which had been secured from different sources. They were isolated originally from hemorrhagic septicemias in various species of animals. The results presented in this report apply only to the typical strains of animal origin and not to other species at times included in this genus.

The basal medium consisted of a 0.5% solution of hydrolyzed purified gelatin to which was added a supplement of 8 amino acids, 0.3% glucose, 0.5% NaCl, 0.2% K_2HPO_4 , 0.005% $MgSO_4$ and 0.001% $CaCl_2$. To this was added 1 cc of Hoagland salt mixture per liter of medium. The amino acid supplement consisted of 20 mg each of valine, tyrosine, tryptophane, cystine, methionine and histidine and 15 mg each of serine and threonine per liter. The medium was adjusted to pH 7.0 with N NaOH solution and tubed in 5 cc quantities.

The accessory growth factors were sterilized by filtration and added aseptically to the basal medium. In the first tests a mixture of known substances was used on the assumption that perhaps some of them might be required by these organisms. This mixture consisted of nicotinamide, diphosphopyridine nucleotide (cozymase), thiamine, thiamine diphosphate (cocarboxylase), riboflavin, beta-alanine, pantothenic acid, vitamin B_6 hydrochloride, nicotinamide methiodide, inositol, glutamine and sodium pyrophosphate.

The *Pasteurella* strains did not develop in the basal medium. Upon addition of the accessory mixture most of the cultures developed readily. Evidently one or more of the added factors was needed by these types. By simplifying the accessory mixture it was found that nicotinamide (or cozymase) and pantothenic acid were required for growth. Neither alone was effective. Two samples of pantothenic acid were used. Results obtained with one sample of 20% purity were duplicated with another of 70% purity. Pantothenic acid could not be replaced by beta-alanine. Tests with several characteristic strains showed that continuous cultivation through successive transplants was accomplished readily in the presence of nicotinamide and pantothenic acid.

Thirteen of the 17 cultures gave results essentially similar to those of *P. avicida* and *P. bovisseptica* I shown in Table I. The other 4 cultures produced a scantier though still distinct growth, indicating that other factors or conditions were needed for ready cell multiplication. It was found that addition of the butyl factor for *Clostridia*¹ (probably biotin²) caused prompt and vigorous growth of

¹ McDaniel, L. E., Woolley, D. W., and Peterson, W. H., *J. Bact.*, 1939, **37**, 259; Woolley, D. W., McDaniel, L. E., and Peterson, W. H., *J. Biol. Chem.*, 1939, **131**, 381.

² Snell, E. E., and Williams, R. J., *J. Am. Chem. Soc.*, 1939, **61**, 3594.

TABLE I.
Effect of Growth Factors upon Development of Several *Pasteurella* Species of Animal Origin.

Basal medium with addition of:	Amt added, μ g per cc of medium	<i>Pasteurella</i>					
		aviceida		bovisseptica I		bovisseptica 18	
		days*		days		days	
		1	2	1	2	1	2
Nothing (control)	—	—	—	—	—	—	—
Nicotinamide	0.1	—	—	—	—	—	—
Pantothenic acid	0.1	—	—	—	—	—	—
Nicotinamide plus pantothenic acid	0.1 each	+++	+++	+++	+++	+	+
Nicotinamide plus beta-alanine	0.1 each	—	—	—	—	—	—
Butyl factor	0.15	—	—	—	—	—	—
Nicotinamide plus pantothenic acid	0.1	—	—	—	—	—	—
plus butyl factor	0.15	+++	+++	+++	+++	++	+++

— = No visible growth, + = very light turbidity just at point of visibility,
+ to +++ = light to pronounced turbidity.

*All cultures were held for 10 days at 37° and observed at frequent intervals.
Usually there was no change after the second day.

3 of the remaining 4 cultures. The growth of *P. bovisseptica* 18 (Table I) is an example of the effect of addition of butyl factor.

On substitution of a mixture of 18 amino acids for the hydrolyzed gelatin solution it was found that with but two exceptions all of the cultures could be grown in the presence of nicotinamide, pantothenic acid and the butyl factor. As far as we are aware cultivation of these organisms in an amino acid medium with the addition of known accessory substances has not hitherto been accomplished.

11359

Normal and Abnormal Prothrombin Levels.

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While low fibrinogen levels have been reported in pernicious anemia, scurvy, pellagra, acute yellow atrophy, and myeloid dyscrasias, there are not available adequate data as to the prothrombin levels of such diseases in man. Quick¹ postulates that the blood in acute yellow atrophy of the liver may be as deficient in

¹ Quick, A. J., *Am. J. Med. Sci.*, 1940, **199**, 123.

prothrombin as in fibrinogen, and regards it highly improbable that a prothrombin deficiency can be produced in the adult by dietary means. It must be remembered that pellagra and sprue are both commonly associated with rather characteristic dietary habits, and evidence has already been offered for prothrombin deficiency in sprue,² and that for hypoprothrombinemia in the absence of jaundice in man due to inadequate vitamin K intake.³ Likewise, there exist bone marrow and intestinal relationships in sprue, pernicious anemia and pellagra.

The present report was undertaken on a small series of cases to determine the average normal limits of prothrombin clotting time in normal adult subjects and in treated and untreated patients suffering from unrelated diseases. The amount of prothrombin was estimated by the methods of Quick^{4, 5} and Warner,⁶ and compared with the prothrombin index of whole blood. A total of 94 clinic and hospital patients, and 17 adult normals were observed over a period of 3 months. Six tests were performed on each concentration of 10% and 5% thromboplastin, with blood taken from the median basilic vein. The potency of the thromboplastin was estimated on the normal adults and was carefully prepared. The data are expressed in the number of seconds required for the clot formation to appear at 37°C with each lot of freshly prepared thromboplastin.

It has been shown by previous investigators that the normal prothrombin time is 16 to 19,⁴ 20,⁸ and 25⁷ sec. with an error of only 5%. Warner, *et al.*, with a plasma dilution method to evaluate the deficiency in prothrombin units have an approximate variation of less than 5%. It seems significant to compare the 3 tests for an index of normal with fresh blood and plasma under ideal conditions.

Table I illustrates the average values for prothrombin clotting time in normal and pathologic patients.

Patients with undiagnosed blood dyscrasias were omitted from the final series. Although the experimental error in the series by the wet and dry methods occasionally exceeded 5-10%, the results none-

² Clark, R. X., Dixon, C. F., Butt, H. R., and Snell, A. M., *Proc. Staff Meet. Mayo Clin.*, 1939, **14**, 107.

³ Kark, R., and Lozner, E. L., *Lancet*, 1939, **2**, 1162.

⁴ Quick, A. J., *Am. J. Med. Sci.*, 1935, **190**, 501.

⁵ Quick, A. J., *Am. J. Physiol.*, 1936, **114**, 282.

⁶ Warner, E. D., Brinkhous, K. M., and Smith, H. P., *Am. J. Physiol.*, 1936, **114**, 667.

⁷ Aggeler, P. M., and Lucia, S. P., *Proc. Soc. Exp. Biol. and Med.*, 1938, **38**, 11.

⁸ Kato, K., and Poncher, H., *J. A. M. A.*, 1940, **114**, 9, 749.

TABLE I.

No. of cases	Diagnosis	Sec. with thromboplastin		Whole blood index	% of normal (Warner)
		10% Wet	5% Dry		
8	Sprue	38	30	1.0	88
2	Thrombocytopenic purpura	39	32	1+	80
6	Polycythemia	29	30	1.0	86
3	Microcytosis	28	25	1.0	92
5	Acute pernicious anemia (untreated)	30	23	1.0	96
12	Pernicious anemia with cord lesions	27	25	1.0	90
16	Pernicious anemia without cord lesions (treated)	27	30	1.0	82
9	Alcoholic polyneuritis	33	30	1+	82
1	Sprue (relapsing)	60	31	1+	76
1	Parathyroidectomy (under treatment)	20	29	1.0	100
4	Pellagra with alcoholism	29	26	1.0	94
3	Tabes with malaria	30	30	1.0	86
1	Partial gastrectomy (Ca)	28	32	1+	82
5	CNS Lues with malaria	32	28	1.0	89
3	CNS Lues without malaria	30	32	1+	84
4	Sickle cell anemia	23	19	1.0	92
1	Lymphogranuloma inguinale	26	21	1.0	96
3	Aplastic anemia	30	33	1+	78
2	Diabetes mellitus	25	27	1.0	90
1	Splenectomy	34	30	1.0	86
2	Hodgkins Disease	32	29	1.0	86
2	Brucellosis	32	26	1.0	92
17	Normal	26	28	1.0	94

theless, under the ideal conditions show that the clotting time of prothrombin in the normal adult ranges up to 30 sec. as the upper limit with a prothrombin index of 1.0 for whole blood clotting. If 25-30 sec. is taken as the upper limit of normal, closer correlation may be found to exist with the other tests. The recent report of Kato and Poncher using the microprothrombin test of Kato⁸ on mature and immature infants, shows that the most mature infants likewise fall in the 25-30 sec. range. This seems to substantiate the results reported here.

At times it is difficult to account for the high readings given by dry thromboplastin, and low readings or *vice versa* from the wet solution prepared from the same source, with more uniform results occurring in this series with the dried material, if the only variable factor is the quality and amount of the prothrombin of the test plasma. Comparison of the prothrombin index of whole blood yields correlation to the studies reported here. Grossly abnormal tests if repeated will establish the general average range which has been observed to be 25 sec. plus or minus in the pathologic cases reported here and not above 30 sec.

None of the patients received synthetic vitamin K in therapy

and depended upon its content in their food. Nicotinic acid and vitamins B₁ and riboflavin were given to the pellagra patients. Alcohol-polyneuritis patients received vitamins B₁ and riboflavin and adequate food.

The sprue patients are interesting, since none of them received synthetic vitamin K and maintained adequate prothrombin levels. A case of sprue (relapsing) with marked diarrhea showed an almost normal prothrombin level. The sprue cases received liver extract and yeast as did all pernicious anemias.

Despite the fact that prolonged readings have been observed in individual cases of pernicious anemias the general average reveals no marked deficiency of prothrombin. This apparently holds true for pellagra and polycythemia, treated and untreated. In a case of aplastic anemia following anti-leukemic therapy, the prothrombin level was slightly reduced. While none of these patients enjoy maximum health, it is significant that an approximately normal or slight diminution of the prothrombin level is found even during characteristic exacerbations of their diseases. The whole blood clotting index may even be less than 1.0, illustrating that the volume per volume content of prothrombin in whole blood and plasma is not very significant.

Conclusions. (1) From the data presented the normal range of prothrombin-clotting time has been observed to be from 25 to 30 sec. Values above 30 sec have given a whole blood index of 1.0 plus. These results yield closer correlation to other methods for estimating the prothrombin level. (2) Treated and untreated patients with pernicious anemia, pellagra and other pathologic conditions have similar normal prothrombin values. Treated sprue patients gave similar results. In a case of sprue (relapsing) with persistent diarrhea, the prothrombin level was found to be almost normal. (3) When abnormal variations occur in wet or dry readings, it may be more accurate to depend upon the whole blood clotting index.

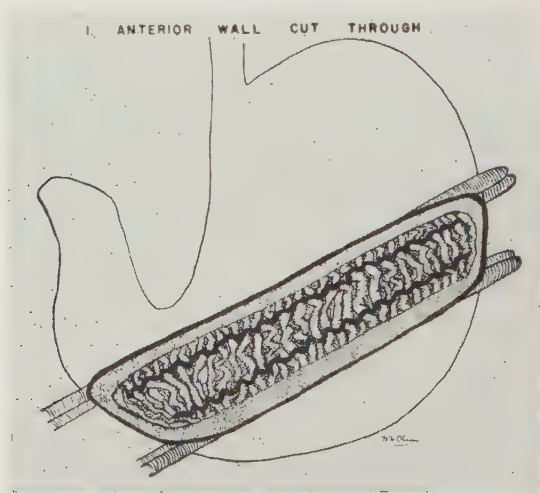
A New and Simple Method for Preparing Large Pavlov Pouches.*

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*From the Department of Gastro-Intestinal Research, Michael Reese Hospital,
Chicago.*

The original method of Pavlov for the preparation of Pavlov pouches in dogs has been criticized recently by Hollander and Jeremin.¹ They devised a new technic in which practically the entire vagal supply to the pouch was left intact. Their method yields excellent pouches, but is difficult and time-consuming. In our hands the eversion of the entire stomach through a small incision seemed to produce marked surgical shock, and increased greatly the danger of peritoneal contamination. Suturing of the mucosa was difficult and perforation between main stomach and pouch likely to occur. We therefore devised a simpler operation which yields big pouches with large amounts of secretion, which we feel worthwhile to make known to other workers in this field.

Procedure. Dogs were fasted for 24 hours and anesthetized with morphine-atropine and ether, or sodium pentobarbital. The stomach was exposed through a left rectus incision. The blood vessels at

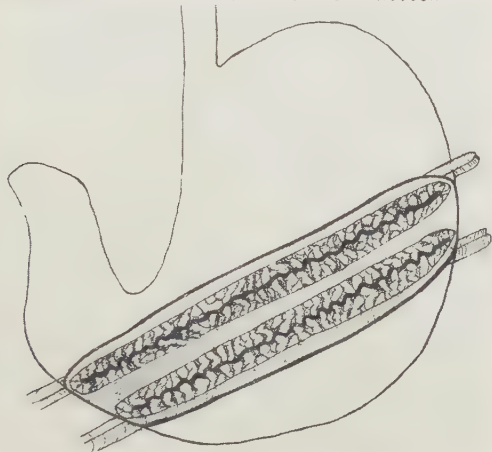


* Aided by the A. B. Kuppenheimer Fund.

¹ Jeremin, E. E., and Hollander, F., *PROC. SOC. EXP. BIOL. AND MED.*, 1938, **38**, 139; Hollander, F., and Jeremin, E. E., *PROC. SOC. EXP. BIOL. AND MED.*, 1938, **39**, 87.

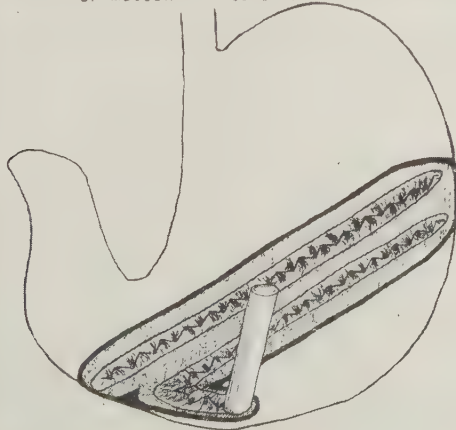
the greater curvature, below the angulus, were ligated and cut. Rubber-covered elastic clamps were put across the lower one-third of the fundus (Fig. 1). A straight incision through the entire anterior wall was performed (lately we cut only $4/5$ of the anterior wall). The mucosa of the posterior wall was cut and about 1 cm was dissected away from the muscularis on each side of the incision (Fig. 2).

2 SEPARATION & CUTTING OF POSTERIOR MUCOSA

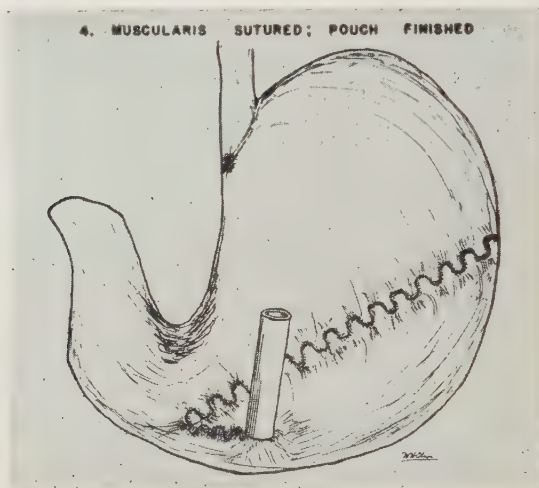


The main stomach was closed by an inverting running suture of the mucosa. The mucosa of the pouch was similarly closed, beginning at the upper end of the incision. The elastic clamp was removed, when about $1/2$ of the pouch had been closed. At the lower pole of the

3. MUCOSA SUTURED



pouch a brass cannula (recently stainless steel) was inserted, and the submucosal suture carried in such a way that an angle was formed (Fig. 3). This is important because it avoids perforation between pouch and main stomach by the round base plate of the cannula. Muscularis of pouch and main stomach were united by Lembert suture (Fig. 4). Omentum was wrapped round the base of the



cannula. A small skin incision was made to the right of the mid-line at the height of the greater gastric curvature. A sharply pointed trocar with sleeve was pushed through this opening, the trocar withdrawn, the cannula pushed into the sleeve and the latter withdrawn. Iodized gauze was placed round the external base of the cannula and a collar attached to it to prevent its slipping back into the pouch. The abdomen was closed in the usual way. Twenty-four hours after operation the iodized gauze was taken off, and the collar moved up, in order to prevent pressure on the skin. The wounds were washed with hydrogen peroxide. One to 2 weeks after operation the dogs were ready for use. Active appetite secretion and immediate response to a meal characterized them as Pavlov pouches, *i. e.*, pouches with good vagal supply. Intravital and postmortem inspection of a number of these pouches showed no irritating effects of the metal cannula. Strong adhesions had formed between the neck of the pouch and the parietal peritoneum round the cannula.

Secretion is collected in 2 ounce glass bottles with screw cap. A collar is soldered on top of the cap, which is attached to the cannula by a screw. The weight of the bottle is supported by straps attached to the screw cap, which are fastened over the back of the animal.

TABLE I.
Male Dog, 25 kg, 30-Min. Samples.

Volume cc	Acidity clinical units	
	Free	Total
0.2	0	7
	Fed 200 g Pard, 200 cc water	
35	140	154
45	150	161
48	149	161
53	152	165
61	157	169
45	154	165
42	158	169
30	149	158
23	162	172
11	132	146

The secretion is water clear. An example of secretion to a meal consisting of $\frac{1}{2}$ pound of Pard dog food with 200 cc of water is given below.

Table I demonstrates that the pouch in the fasting dog has a minimal amount of secretion (0.07 cc per minute), no free and a minimal amount of total acid. Following a meal, free and total acid reach high values within the first half-hour and stay near the physiological maximum of acid secretion for $4\frac{1}{2}$ hours. The total volume of fluid secreted during this period amounted to 382 cc, *i. e.*, 1.4 cc per minute. Likewise the response of these pouches to histamine is excellent.

The posterior wall of the pouch has a complete vagal supply. The greater part ($\frac{4}{5}$) of the vagus branches of the anterior wall has been cut. In view of the excellent secretory performance of the pouch one can assume that the submucous plexus of the anterior wall receives sufficient vagal innervation from the remaining $\frac{1}{5}$ of its connection with the musculature of the anterior wall, and from complete muscular bridge with the posterior wall of the main stomach. Dogs with such pouches have been in use in this laboratory for one year, and their pouch secretion has not changed. They are in fine physical condition and have no erosions or irritation round their cannula.

Summary. A new, simple and efficient method for preparation of Pavlov pouches is described, leaving intact more than 50% of the normal vagus supply to the pouch. The pouch has hardly any basal (fasting) secretion, with no free and very little combined acid. These pouches respond with maximal secretion, both volume and acidity, to food and histamine.

Experimental Cinchophen Ulcer.*

FRANK NEUWELT AND H. NECHELES.

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The cinchophen ulcer has been studied extensively since the first observations of Churchill and Van Wagoner.¹ Stalker, Bollman and Mann have examined the problem of the cinchophen ulcer most thoroughly, and have reported their findings in several recent publications.^{2, 3, 4} Our laboratory has been interested in this problem for evaluation of ulcer therapy, and herein are presented our observations, some of which are at variance with previous findings.

Chronic Feeding Experiments. Seven dogs weighing from 8 to 14 kg were fed a diet of kitchen scraps (without bones), white bread, yeast, bone meal, salt, and cod liver oil to which was added daily 1 to 5 g of cinchophen.†

Results. A 9.5 kg female white Spitz died after receiving 1 g of cinchophen daily for 12 days. Necropsy revealed a large perforated ulcer surrounded by smaller ulcerations on the proximal posterior aspect of the pyloric canal. A second white Spitz received 3 g of cinchophen daily for 10 days. The animal's weight declined from 8 to 6 kg, and it was found dead on the morning of the 11th day. Autopsy showed empyema of the chest and numerous small ulcerations of stomach and duodenum. One large shallow ulcer 3 cm in diameter was situated at the entrance of the pylorus. A third white Spitz (11 kg) received 25 g of cinchophen during a period of 20 days. The animal lost weight, refused to eat and became cachetic, but stools did not become tarry. Sixteen days after stopping the administration of cinchophen the animal died. At necropsy a number of acute small ulcers were found in the antral region of the stomach and in the first portion of the duodenum. A large,

* Aided by the A. B. Kuppenheimer Fund.

¹ Churchill, T. P., and Van Wagoner, F. H., *Proc. Soc. Exp. Biol. and Med.*, 1931, **28**, 581.

² Stalker, L. K., Bollman, J. L., and Mann, F. C., *Arch. Surg.*, 1937, **35**, 290, 294.

³ Bollman, J. L., Stalker, L. K., and Mann, F. C., *Arch. Int. Med.*, 1938, **61**, 119.

⁴ Stalker, L. K., Bollman, J. L., and Mann, F. C., *Arch. Surg.*, 1937, **34**, 1172, 1174, 1176.

† We are obliged to Dr. D. A. Bryce of the Calco Chemical Company for supplying the cinchophen.

deep chronic ulcer 2 x 2 cm in diameter was found near the pylorus on the posterior wall of the stomach.

The following 4 animals were of mixed breed. The fourth animal (9 kg) was fed a total of 14 g of cinchophen over a period of 14 days; no tarry stools, anorexia or loss of weight appeared. One month later 138 g of cinchophen were fed over a period of 48 days, at the end of which laparotomy with opening of stomach and duodenum failed to disclose peptic ulcerations. The stools were persistently negative for blood. The fifth dog (13.5 kg) was fed 4 g of cinchophen in 4 days. He then had complete anorexia but no tarry stools. We suspected a perforating, non-bleeding ulcer and laparotomy was performed. Stomach and duodenum were opened, but no ulceration, gastritis or duodenitis observed. A few days later the animal died of pneumonia which probably had begun earlier and had caused anorexia. Autopsy did not reveal gastrointestinal pathology. In dogs dying from pneumonia or distemper, gastritis and erosions are not infrequent. The sixth and seventh dog (12 and 14 kg) received 19 and 74 g of cinchophen in 19 and 24 days respectively. No tarry stools or other symptoms of ulcer were noted in either of these dogs.

It has been shown that the development of ulcer from oral administration of cinchophen is variable from the point of view of onset. Practically every experimenter in this field has found a small percentage of animals in which no ulcers developed under administration of cinchophen or certain other drugs even in high doses. In a comparable series of experiments by Stalker, Bollman and Mann ulcer developed in 11 out of 12 dogs that were not fed bone, in an average of 18 days after the administration of 36 g of cinchophen.² In our series 3 dogs died and peptic ulcers were found at necropsy; the remaining 4 did not show evidence of ulceration during periods of administration of the drug of 4, 19, 24, and 47 days, although daily doses of approximately 3 and 4 g were given to the last 2 dogs. On the other hand the 3 dogs that died had been fed smaller doses of the drug over shorter periods of time (1, 2 and 3 g per day over 12, 20 and 10 days respectively). It is, therefore, of interest to point out that these 3 animals were white Spitzes. Perhaps this species of dog is more susceptible to cinchophen ulcers than others. A second point is that one of these animals died 16 days after the last dose of cinchophen and stomach and duodenum contained a number of acute ulcers, as well as one large chronic prepyloric ulceration. Stalker, Bollman and Mann² found that no new ulcers are formed and that chronic ones begin to heal practically immediately

after cessation of administration of the drug. Hence, in the above dog, the etiologic cause for the acute and chronic ulcers remains open.

Effect of Cinchophen on Pouch Secretion. Four dogs with Heidenhain pouches, drained by a metal cannula were used. Their body weights were from 15 to 25 kg, with an average of 22 kg. Twice a week secretion of the pouch was stimulated by subcutaneous injection of 1 mg of histamine acid phosphate every half-hour for a period of 3 hours. This method was chosen in order to establish the maximum secretory capacity of the oxyntic cells. Free and total acidity were titrated against dimethyl-amino-azobenzene and phenolphthalein. Control studies of gastric secretion were performed for several weeks, and cinchophen was administered intravenously as the neutral sodium salt, so as to be certain that the full dose was given and retained. The dose was 1 to 2 g per injection, given 4 to 6 times a week. Total amounts of drug administered to the 4 dogs were as follows: 32 g in 16 days, 32 g in 39 days, 12 g in 15 days, 50 g in 25 days, and one month later 28 g in 22 days. Several of the animals did not bleed from their pouch until 12 or more injections of cinchophen had been given; at that time they showed symptoms of early gastritis, such as occasional tarry stools, vomiting and bleeding from the pouch, but analyses of histamine-stimulated secretion showed no increase in volume secretion at any time during the period of administration of the drug. As a matter of fact the amount of secretion decreased somewhat and became irregular. Free and total acidity also tended to diminish and fluctuate erratically, independent of occasional bleeding from the pouch. In one dog the highest values for free and total acidity were observed when bleeding began, just one day before death. Autopsy revealed peritonitis from perforated duodenal ulcer, just beyond the pyloric ring. Gastritis was present in main stomach and pouch; this was the only animal in the series which died or perforated.

Stalker, Bollman and Mann⁴ found an increased volume secretion in their pouch dogs following administration of cinchophen when symptoms of early gastritis, such as tarry stools and occasional vomiting appeared. When administration of cinchophen was discontinued, a gradual decrease in the amount of gastric secretion to the normal level occurred within 2 weeks. They employed food as secretagogue for their pouch dogs and histamine for their dogs with intact stomachs. Gastric juice was collected continuously from their pouch dogs, but secretion tests on their dogs with intact stomachs was done every second or third day. The dogs of the latter group

(17 kg average weight) received one dose of 1.5 mg of histamine subcutaneously. Yet the results obtained in both groups of animals were identical with regard to increased volume of secretion. The administration of 0.5 g or more of cinchophen caused evidence of gastritis in every dog within 8 hours after the initial dose; when the dose was 1 g or more daily, ulcers developed in from 2-5 days and usually perforated.⁴

The discrepancy between our results and those of Stalker, Bollman and Mann can hardly be found in the fact that our technical procedures were different. Our pouch dogs (22 kg average weight) received 1 mg of histamine every half hour for 3 hours (total of 6 mg). We feel that an augmented volume of secretion would certainly have been found by this method. Our pouch dogs received roughly similar individual doses of cinchophen by vein and for longer periods than did the pouch dogs of Stalker, Bollman and Mann⁴ by mouth, *i. e.*, larger total amounts of the drug, and yet only one animal died from perforated gastric ulcer while the rest survived. It has been shown that the drug acts after absorption, and not locally,⁵ or after excretion into the stomach from the blood.² Therefore one cannot assume that oral or intravenous administration of cinchophen should have different effects. Furthermore, vomiting may cause loss of orally administered drug. It may be that our preparation of cinchophen varies in some way from that of the above and other workers.[‡]

Summary and Conclusions. 1. White Spitz dogs seem more prone to develop cinchophen ulcers than other species of dogs. 2. Heidenhain pouch dogs receiving cinchophen intravenously show no increase in volume secretion under histamine stimulation.

⁵ Churchill, T. P., and Manshardt, D. O. *Proc. Soc. Exp. Biol. and Med.*, 1933, **30**, 825.

[‡] Cinchophen Calco, Lot No. 24331 and 29056 were used.

Vago-Neurohypophysial Pressor Reflex.

D. G. SATTLER. (Introduced by W. R. Ingram.)

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It has been shown repeatedly that interruption of the supraoptico-hypophysial tract causes atrophy of the neurohypophysis with certain cellular alterations. If sufficient neurohypophysis is deprived of its nerve supply in this way, diabetes insipidus ensues (Fisher, Ingram and Ranson¹). These atrophic neurohypophyses have been found to be lacking in the hormonal substances produced by the normal gland, and it is generally held that diabetes insipidus is due to lack of an antidiuretic hormone. The question remains, is the innervation of the neurohypophysis a trophic one, or may it participate in the regulation of the functions of the latter? There is some evidence for neurogenic control of the neurohypophysis.

Theobald and Verney,² and Pickford³ have advanced indirect evidence to show that humoral and neural influences may cause the neurohypophysis to increase its output of antidiuretic substance. Haterius⁴ has confirmed this and added more direct evidence for participation by the pituitary. Gilman and Goodman⁵ found antidiuretic substance in the urine of dehydrated normal rats, as did Boylston and Ivy,⁶ the latter pointing out the similarity between the action of this antidiuretic substance and that of pitressin. Gilman and Goodman could not obtain similar results with dehydrated hypophysectomized rats. Ingram, Ladd and Benbow⁷ have offered evidence that while appreciable amounts of antidiuretic substance are excreted by normal cats in a state of dehydration, dehydrated cats with diabetes insipidus do not excrete such material. Walker,⁸ however, did not obtain such results. Gersh⁹ reports that "paren-

¹ Fisher, C., Ingram, W. R., and Ranson, S. W., *Diabetes Insipidus*, Edwards Brothers, Inc., 1938.

² Theobald, G. W., and Verney, E. B., *J. Physiol.*, 1935, **83**, 341.

³ Pickford, M., *J. Physiol.*, 1939, **95**, 226.

⁴ Haterius, H. O., *Am. J. Physiol.*, 1940, **128**, 506.

⁵ Gilman, A., and Goodman, L. S., *J. Physiol.*, 1937, **90**, 113.

⁶ Boylston, C. A., and Ivy, A. C., *PROC. SOC. EXP. BIOL. AND MED.*, 1938, **38**, 644.

⁷ Ingram, W. R., Ladd, L., and Benbow, J. T., *Am. J. Physiol.*, 1939, **127**, 544.

⁸ Walker, A. M., *Am. J. Physiol.*, 1939, **127**, 519.

⁹ Gersh, I., *Am. J. Anat.*, 1939, **64**, 407.

chymatous glandular cells" of the neurohypophysis of rats increase in size and number in dehydration and at parturition.

Chang, Lim, *et al.*,^{10, 11} have reported a series of interesting observations made upon experimental animals in which the only connection between head and body was vascular. Stimulation of the central end of the cut vagus in such dogs caused characteristic elevations of blood pressure. Since acute hypophysectomy abolished this response, they ascribed this reflex to a liberation of a pressor principle from the neurohypophysis, indicating that the integrity of the reflex arc depended upon the presence of an intact supraoptico-hypophysial tract and anatomically obscure central intermediary neurons. In similar experiments increased production of oxytocic, glucogenic and antidiuretic substances was also described. These results are of such significance as to warrant confirmation. This communication deals with an attempt to confirm certain findings of these workers, and with further data from experiments carried out on dogs with chronically denervated neurohypophyses. The latter experiments were designed to rule out errors which may conceivably be introduced by the trauma and shock contingent upon acute hypophysectomy.

Methods. In all experiments nembutal anesthesia was given intravenously. Blood pressure was recorded from the femoral artery. After isolation of the carotids, jugulars, and vagi, and cannulation of the trachea, all other neck structures were crushed in a specially constructed vise according to the methods of Chang, *et al.* The artificial respiration rate was 8 respirations per minute. The blood pressure fall consequent to cord crushing was combated by the intravenous administration of Ringer's solution and 5 mg doses of ephedrine. By these means it was found possible to maintain the blood pressure at or above 70 mm Hg; experiments in which the basal pressure was lower than this level were discarded. Destruction of the spinal cord was checked at autopsy.

Results. 1. *Acute experiments.* After the technic of preparing such an animal was sufficiently developed, it was found that the rise in blood pressure on stimulation of the central end of the severed vagus was quite easily obtainable. In these experiments, acute hypophysectomy was carried out through an opening in the roof of the mouth so as not to disturb other intracranial structures. The

¹⁰ Chang, H. C., Chia, K. F., Hsu, C. H., and Lim, R. K. S., *Chin. J. Physiol.*, 1937, **12**, 309.

¹¹ Chang, H. C., Lim, R. K. S., Lu, Y. M., Wang, C. C., and Wang, K. J., *Chin. J. Physiol.*, 1938, **13**, 269.

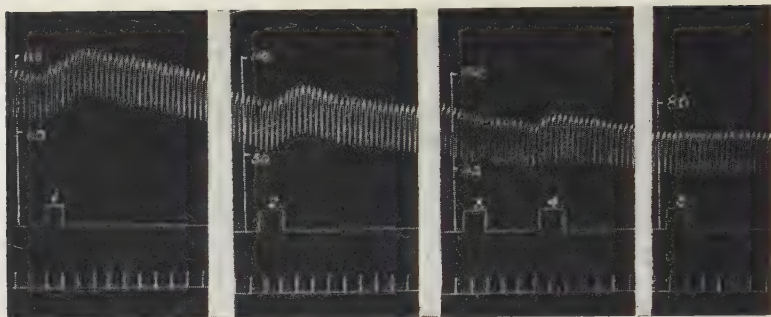


FIG. 1.

Blood pressure tracings from a dog with the cervical spinal cord crushed. Responses to stimulation of the central vagus stump, with the effect of cervical sympathetic ganglionectomy and hypophysectomy.

completeness of posterior lobe resection was confirmed by careful autopsy examination.

The results of a typical experiment are illustrated in Fig. 1. At 1, faradic stimulation (coil at 5 cm) of the left vago-sympathetic trunk for 45 sec. Note the sudden pressor effect upon which a second mild pressor phase is superimposed. Between 1 and 2 the left superior cervical sympathetic ganglion was removed. At 2, stimulation of the central end of the left vagus for 45 seconds. After a 30-second delay a rise in pressure occurs. Between 2 and 3 the hypophysis was removed through a previously made opening in the roof of the mouth. At 3, stimulation of the central end of the left vagus for 45 seconds. No response. At 4, the intact right vago-sympathetic trunk was stimulated for 45 seconds. Sudden onset of pressor response. Between 4 and 5 the right superior cervical sympathetic ganglion was removed. At 5, stimulation of the right vagus caused no response in blood pressure. Such results were obtained in 7 experiments.

Conclusions. In a normal dog, with the spinal cord crushed, stimulation of the central end of the vago-sympathetic trunk causes a pressor effect in the body. This effect seems to be of two components; a sudden rise (sympathetic effect) and a more delayed rise (vagus-neurohypophysis effect). That the elevated pressure resulting from stimulation of the vagus alone is effected through the pituitary is indicated by abolition of the response through hypophysectomy.

This experiment was tried on several cats with similar results but with great variability under the conditions. Technical obstacles made the experiment difficult.

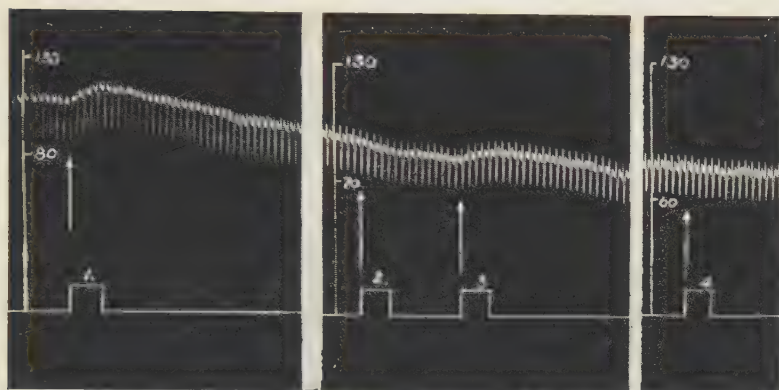


FIG. 2.

Blood pressure tracings from dog 14, with diabetes insipidus and the cervical spinal cord crushed. Responses to stimulation of the central vagus stump before and after cervical sympathetic ganglionectomy.

2. *Experiments with dogs with chronically denervated neurohypophyses.* In these animals the supraoptichypophyseal tract was sectioned in the median eminence, using a subtemporal approach. Criteria for the completeness of the section were the occurrence of marked permanent diabetes insipidus and examination of microscopic sections of the infundibular region. These dogs were used to show further that the pressor response is abolished in the absence of neurohypophyseal innervation, and to rule out any possible error caused by trauma and shock in the acutely hypophysectomized animals.

The following experiment is typical:

Dog No. 14. Before stalk section average urine output was 200 cc (Sp. Gr. 1.032). After section of the stalk average urine output was 2978 cc (Sp. Gr. 1.002). Following the 6th day there was a 4-day normal interphase after which the water intake and urine output rose to the previous high level. On the 16th postoperative day the spinal cord was crushed and stimulations were carried out (Fig. 2). At 1, faradic stimulation of the right vago-sympathetic trunk (coil at 5 cm) for 45 seconds. Immediate onset of a pressor effect. Between 1 and 2 the right superior cervical sympathetic ganglion was removed. At 2, stimulation of the right vagus for 45 seconds caused no change in blood pressure. At 3, stimulation of the left vago-sympathetic trunk for 45 seconds. Immediate pressor effect. Between 3 and 4 the left superior cervical sympathetic ganglion was removed. At 4, stimulation of the left vagus for 45 seconds caused no change in the blood pressure.

Similar results were obtained in 3 experiments. In addition a dog

with diabetes insipidus in which cervical sympathetic ganglionectomy was not done yielded only typical sympathetic responses without evidence of vagus pressor responses. In one operated dog with a good transient but not a permanent polyuria, vagoneurohypophysial responses were not obtained; microscopic sections showed extensive but not quite complete degeneration of the supraopticohypophysial system.

Conclusions. Stimulation of the central end of the vago-sympathetic trunk in a dog with diabetes insipidus gives a pressor response. This pressor response is due to stimulation of the sympathetics of the head, since the response is abolished by sympathetic ganglionectomy. In the absence of the supraopticohypophysial connection the vagus-postpituitary reflex is not obtainable even under the best conditions, as when the basal blood pressure level is high.

3. In a number of acute experiments the infundibulum was stimulated directly with weak faradic current. Fine bipolar electrodes were used and introduced manually through a buccal opening. The spinal cords were completely crushed in each of these dogs. Striking elevations in blood pressure were obtained. These experiments supplement those of Clark and Wang¹² in which hypothalamic stimulation produced pressor effects in spinal cats, and offer further indication that the results of these workers were due to activation of the neurohypophysis.

Summary. 1. In dogs with only vascular connections between head and body, stimulation of the central end of the severed vagus causes blood pressure elevations in the body. Acute hypophysectomy abolishes this reflex. 2. This reflex cannot be obtained in preparations with chronic diabetes insipidus caused by interruption of the supraopticohypophysial tract. This rules out possible error due to shock, trauma, etc., consequent to acute hypophysectomy. 3. These results add to the evidence found elsewhere that the neurohypophysis is subject to nervous control mediated by the supraopticohypophysial tract.

¹² Clark, G., and Wang, S. C., *Am. J. Physiol.*, 1939, **127**, 597.

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A Modified Method for the Preparation of Renin.

W. D. COLLINGS,* J. W. REMINGTON, H. W. HAYS AND V. A. DRILL. (Introduced by W. W. Swingle.)

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Partially purified renin has been prepared in several laboratories from NaCl or cold acetone extracts of the kidney cortex.¹⁻⁴ Recently modifications in our original extraction procedure² have been introduced which eliminate many of the more cumbersome manipulations involved, with considerable saving of time, without sacrificing the relative high potency of the final product.

Extraction Procedure. Demedullated kidneys are ground, frozen, and, before thawing, reground into 2% NaCl solution (10 liters to 3 kg tissue). It is possible to use fresh, unfrozen kidneys, but the extraction appears to be less complete. After the salt extract has been stored for 24 hours under toluene, the meat sludge is removed by straining and centrifuging (Sharples). The pH is then lowered to 4.5. After an interval of 12 to 24 hours to insure complete precipitation, the heavy precipitate is removed by centrifuging (Sharples) and filtering through Hyflo Super-Cel.† For easier handling in subsequent procedures, the filtrate, adjusted to pH 6.8, is concentrated *in vacuo* (maximum temperature 45°C) to a volume of 1 liter. The concentrate is filtered, 100 g NaCl are added and the pH is lowered to 2.0. The heavy precipitate is removed on a filter cake of Hyflo which is then suspended in 2 liters of water. After the pH is raised to neutrality and the suspension thoroughly mixed by a motor stirrer for about 30 minutes, Hyflo and any insoluble precipitate are filtered off and discarded. The filtrate is saturated with solid NaCl and the pH again lowered to 2.0. The precipitate is removed on a filter cake and redissolved in 500 cc N/10 acetate buffer at pH 5. As before, insoluble residue is discarded. At this stage two lots of extract (each representing 3 kg kidney cortex) are combined in the same 500 cc of buffer solution. Precipitation with

* E. R. Squibb and Sons Fellow in the Biological Sciences.

1 Helmer, O. M., and Page, I. H., *J. Biol. Chem.*, 1939, **127**, 757.

2 Swingle, W. W., Taylor, A. R., Collings, W. D., and Hays, H. W., *Am. J. Physiol.*, 1939, **127**, 768.

3 Hessel, G., *Klin. Woch.*, 1938, **17**, 843.

4 Hill, J. R., and Pickering, G. W., *Clin. Sci.*, 1939, **4**, 207.

† Celite Filter Aid, Johns-Manville Company, New York.

solid ammonium sulfate at 0.4 saturation is made 5 successive times, with the volume of buffer solution reduced at each step, *e. g.*, 500, 400, 300, 200, and 100 cc. The first 4 precipitates are removed on filter cakes, the 5th is removed by centrifuging. The discarded supernatant should be color-free. When the final precipitate is not easily soluble in 40-70 cc water, to give a clear, light amber solution, ammonium sulfate precipitations are repeated from a volume of 50-100 cc. It is important that the volume of buffer solution be kept small, since inactive less soluble globulins are left behind at each filtration. The extract is now dialyzed at 6°C against distilled water. We have found that any precipitate forming in the dialyzer can be discarded as inactive. The final volume of 60-100 cc should have 10-15 mg solids per cc. Where the total solids are higher, further ammonium sulfate precipitations from the dialyzed solutions are usually made. The recovery is not quantitative, but inactive solids are often eliminated by this step.

All pH adjustments are made by 10% HCl or 10% NaOH and are measured with a glass electrode. Stock reagents are kept in the refrigerator, and care is taken to keep the extract chilled as much as possible throughout its preparation.

Dialysis. A circulating dialyzer⁵ in the refrigerator is usually employed. However, no difference in the activity of the final product has been observed when the slower, standing dialysis (cellophane bag in a cylinder of water) is substituted. There is apparently no loss of potency, in the cold, even though dialysis is prolonged 3 to 4 days.

Stability. The extract is stored either frozen or in the lyophile state. The latter material is prepared by the Cryochem process⁶ and stored in the refrigerator. It has shown full activity when tested as much as 10 months after preparation. Frozen extract, especially if melted and refrozen repeatedly, shows a gradual loss of activity.

Preparation of Sterile Renin. The extract can be sterilized by filtering through a Jena glass filter and then rendered lyophile in sterile vials. A Seitz filter adsorbs all the active protein from the acid solution.

Chemical Properties. Renin, as prepared by the method described above, is a pseudo-globulin and is precipitated by 0.38 to 0.41 saturated ammonium sulfate at pH 5, by saturated NaCl, by 0.7 to 1.0 saturated magnesium sulfate and by the various protein precipitants.

⁵ Taylor, A. R., Parpart, A. K., and Ballentine, R., *Ind. and Eng. Chem.*, 1939, **11**, 659.

⁶ Flosdorf, E. W., and Mudd, S., *J. Immunol.*, 1938, **34**, 469.

TABLE I.
Some Color Reactions of Renin.

Reaction	Reactive group	Result
Biuret	peptide linkage	+
Millon	tyrosine	++
Xanthoproteic	benzene nucleus	+
Hopkins-Cole	tryptophane	+
Sullivan's	cysteine, cystine	—
Ehrlich-diazo	histidine, tyrosine	+
Sakaguchi	arginine (guanidine)	+
Molisch	carbohydrates	—
Benzidine	pentoses	—

It will form a picrate and is destroyed rapidly by boiling and by protein denaturants. It is free from carbohydrate and responds to routine color tests as shown in Table I. The test for —SH groupings was negative in both native and denatured (boiled) protein and was absent also after treatment with NaCN. However, renin shows the presence of sulfur after sodium fusion.

Yield. The yield in total solids per kg kidney cortex is fairly constant at 150-180 mg. The variability in activity, however, is large, for reasons at present not known. The renin unit² has been defined as the amount of material, per kg body weight, which will produce a 40 mm rise in the mean blood pressure of the anesthetized dog. In the assay standard, 1 unit was the equivalent of 0.1 mg renin. In 39 successive lots of extract, the rise given by 0.1 mg renin, per kg body weight, has been between the extremes of 13 and 62 mm, with the mean at 34 mm. In other words, the average yield was 1450 units per kg fresh cortex. In our most potent extract (Table II) 1 unit represents 9.6 μ g nitrogen.

TABLE II.
Renin Assay at Dosage of 0.1 mg per kg Body Weight.

Dog No.	1	2	3	4	5	6	7	8	9	10	11	12	Avg
Body wt, kg	9.7	9.5	13.1	12.5	7.5	9.5	7.6	10.7	12.5	11.6	12.6	9.4	10.5
Initial B.P.,* mm Hg	108	114	115	103	88	89	122	93	146	95	125	96	108
Peak B.P., mm Hg	175	165	177	209	174	139	178	139	199	156	172	157	170
Rise in B.P., mm Hg	67	51	62	106	86	50	56	46	53	61	47	61	62.2

*B.P. readings are mean pressures obtained by intra-arterial needle puncture.

Destruction of Ascorbic Acid in the Rumen of the Dairy Cow.*

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Dutcher and coworkers,¹ Hart, Steenbock and Ellis,² and Hess, Unger and Supplee³ presented evidence to show that the diet of a dairy cow influenced the antiscorbutic potency of the milk produced. Using guinea pigs to test the antiscorbutic potency of the ration which was fed and to assay the milk which was produced, each of these groups of workers found that milk obtained from cows on a vitamin-rich ration was definitely superior in antiscorbutic value to the milk derived from cows on a vitamin-poor diet. These findings, though widely accepted, were disputed by Hughes and coworkers,⁴ who concluded from a series of experiments that the ration received by cows had no influence on the antiscorbutic property of their milk.

Since the development of chemical methods for the quantitative determination of the antiscorbutic factor, which was shown to be ascorbic acid, differences of opinion have arisen concerning the factors which have the greatest influence on the amount of vitamin C in milk. It is now generally agreed, however, that the vitamin C content of milk is independent of the season of the year and the ration of the cow.⁵ This fact has led to the present investigation of the fate of ingested ascorbic acid in the cow.

A rumen fistula was made in a Holstein cow. Experiments were performed in which this cow was fed (A) 100 g (2,000,000 International Units) and (B) 150 g of synthetic ascorbic acid mixed with corn silage; 100 g of ascorbic acid were also placed directly in the rumen through the fistula opening.

Similar results were obtained in all of the experiments. No increase was observed in the ascorbic acid values of the blood plasma and of the milk when compared with those values obtained while

* Authorized for publication on March 19, 1940, as paper No. 963 in the Journal Series of the Pennsylvania Agricultural Experiment Station.

¹ Dutcher, R. A., Eckles, C. H., Dahle, C. D., Mead, S. W., and Schaffer, O. G., *J. Biol. Chem.*, 1920, **45**, 119.

² Hart, E. B., Steenbock, H., and Ellis, N. R., *J. Biol. Chem.*, 1920, **42**, 383.

³ Hess, A. F., Unger, L. J., and Supplee, G. C., *J. Biol. Chem.*, 1920, **45**, 229.

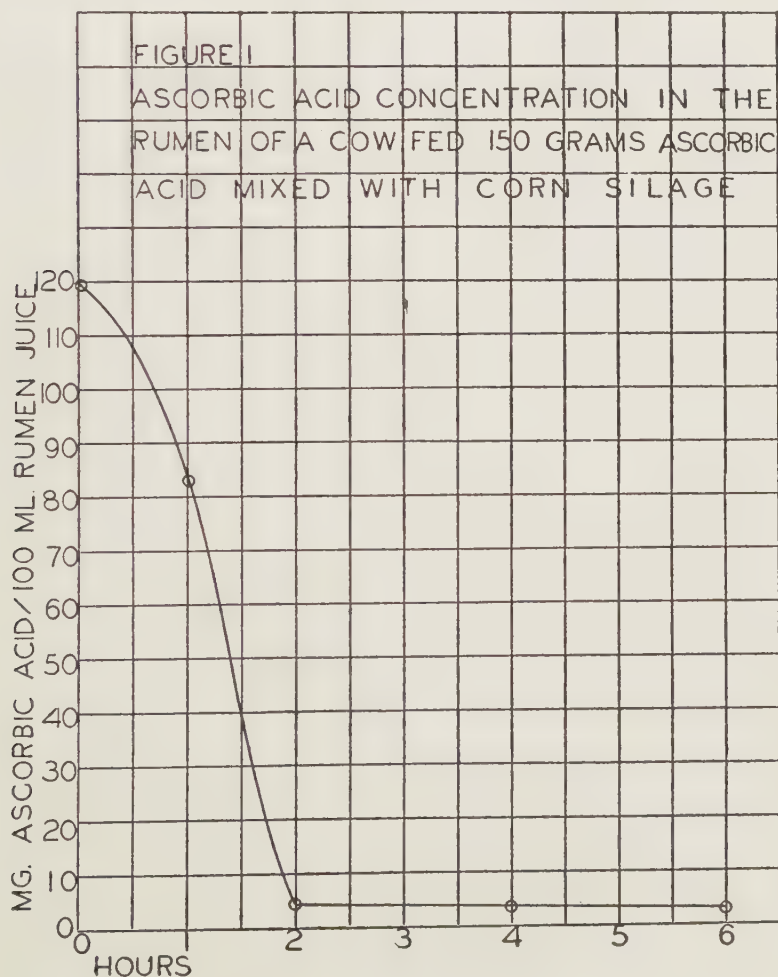
⁴ Hughes, J. S., Fitch, J. B., and Cave, H. W., *J. Biol. Chem.*, 1921, **46**, L.

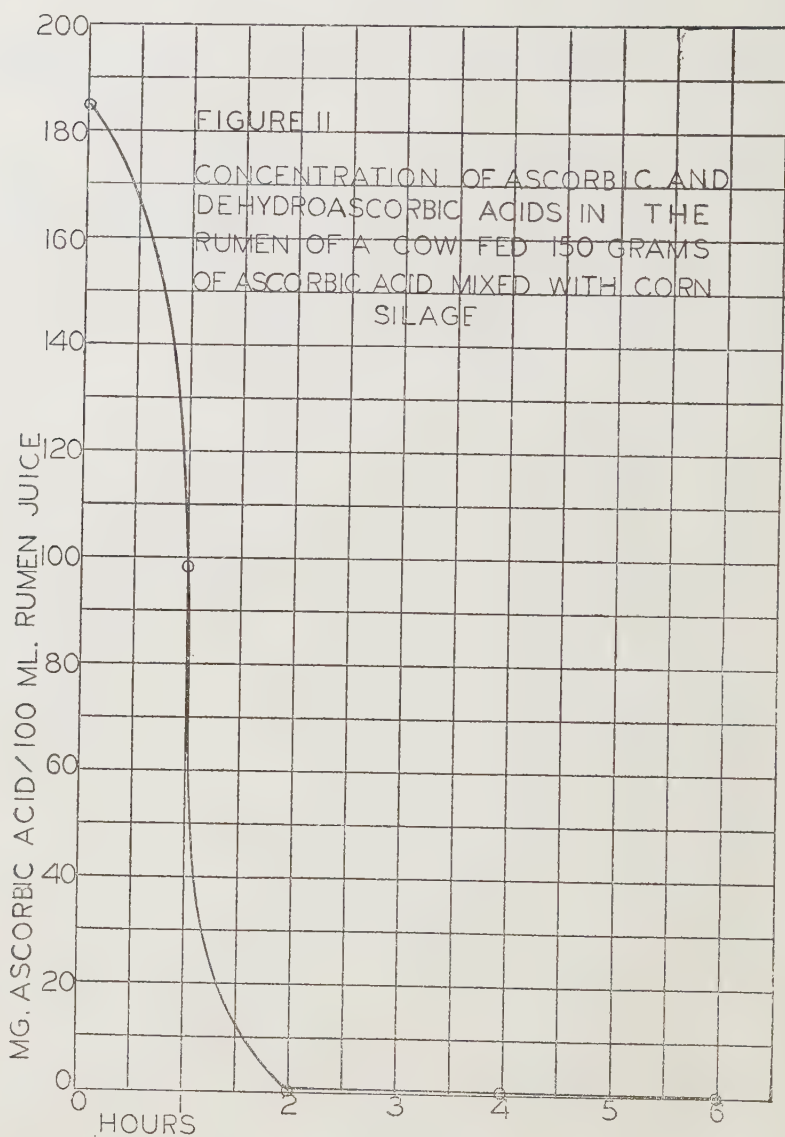
⁵ Kon, S. K., *The Journal of Dairy Research*, 1938, **9**, 242.

the cow was on a standard ration unsupplemented with the vitamin. A slight increase was noticed in the amount of ascorbic acid found in the 24-hour sample of urine for the periods during which the vitamin was administered.

A rapid and pronounced destruction of ascorbic acid in the rumen was demonstrated by removal and analysis of samples of the rumen contents at regular intervals after the cow had been fed (Figs. 1 and 2). Ascorbic acid added to rumen contents *in vitro* and stored in a dark-glass, stoppered bottle at 39°-42°C disappeared at much the same rate as that of the *in vivo* experiments.

These results are not in accord with the conclusions of Riddell and





Whitnah⁶ who suggested that the rapid disappearance of vitamin C from the rumen of a cow fed large amounts of green rye was due to a quick absorption of the vitamin.

In making the above analyses, both the indophenol titration and the Roe furfural method were employed.⁷ The latter method was useful

⁶ Riddell, W. H., and Whitnah, C. H., *J. Dairy Science*, 1938, **21**, 121.

⁷ Roe, J. H., and Hall, J. M., *J. Biol. Chem.*, 1939, **128**, 329.

in detecting dehydroascorbic acid as well as the reduced form of the vitamin.

The authors wish to acknowledge the assistance of Dr. J. F. Shigley, who performed the fistula operation, and to express their appreciation for the generous supply of ascorbic acid furnished by Chas. Pfizer and Company, New York.

11365 P

Effect of Electrotonus on Accommodation in Nerve.

ABRAHAM M. SHANES. (Introduced by Robert Chambers.)

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Hill¹ has proposed an approach to the response of excitable tissues involving two processes, one a rise of the "local potential" and the other a change of threshold called "accommodation", the rates of which are represented by the time constants " k " and " λ ", respectively. Blair² has pointed out some theoretical inadequacies arising from investigations of the effects of electrotonus on rheobase and chronaxie (theoretically $.693k$), but in the absence of similar studies on λ , the extent of such limitations is not clear. Consequently, the present investigation of λ was undertaken.

The technic described by Solandt³ employing exponentially rising currents was used to determine the λ of the sciatic nerves of *Rana pipiens*. The same nonpolarizable electrodes, 2 cm apart, were employed to produce a 2-second electrotonus and to apply the exponential currents. Special precautions were taken to minimize residual and progressive effects. Most experiments were performed at 20°C.

The chief results obtained are summarized in the accompanying figure. The ordinate represents the relative change in λ (*i.e.*, the ratio of λ during electrotonus, λ_e , to λ of the normal nerve, λ_n) and in rheobase (*i.e.*, the rheobase during electrotonus, V_e , divided by its normal value, V_n), while the abscissa is the intensity of electrotonus (E/V_n) in rheobases. It can be seen from the continuous

¹ Hill, A. V., *Proc. Roy. Soc. London*, Ser. B, 1936, **119**, 305.

² Blair, H. A., *Cold Spring Harbor Symposia of Quantitative Biology*, 1936, **4**, 63.

³ Solandt, D. Y., *Proc. Roy. Soc. London*, Ser. B, 1936, **119**, 355.

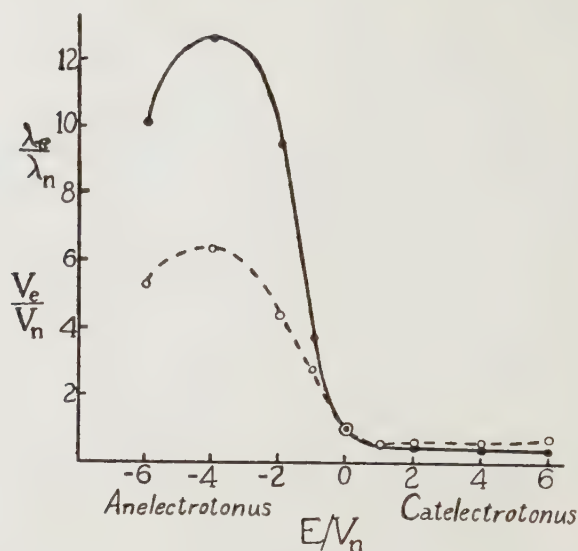


FIG. 1.

curve, which represents the modifications in λ , that λ is increased by anelectrotonus and decreased by catelectrotonus. In none of the 168 measurements of λ during electrotonus was an exception to this found. It is obvious, too, that λ is a continuous function of the electrotonic intensity, a function which is similar to that of rheobase represented by the broken curve.

Such similarity is exactly contrary to the behavior to be expected if alterations of k and λ alone govern the behavior of the nerve. For example, an increase in rheobase is often interpreted as being caused by faster accommodation. Experiments not involving electrotonus have also shown rheobase and λ to behave in such a theoretically unexplainable manner. Thus, unsoaked nerves of summer frogs mounted in a moist chamber at 29°C also exhibited an increase of both rheobase and λ with time; measurements before and after soaking in Ringer's solution often indicated the same. Furthermore, preliminary experiments indicate that although nerves soaked in calcium-rich Ringer's solution show an increase in rheobase, as expected from the concomitant decrease in λ , the order of magnitude of this increase is much larger than theoretically accountable by the change in λ . This excessive alteration in rheobase can be shown to explain the absence in calcium-treated nerves of the initial curvature theoretically predicted for the curve relating the relative thresholds of stimulation and the time constants of exponential current rise (from which λ is determined). The absence of this

curvature was noticed by Solandt, who was unable to account for this divergence from theory.

Comparison of the electrotonic effects which have been described with those obtained by Nivet⁴ for chronaxie and rheobase indicates a possible relationship between λ and k which is contrary to the suggestion of their independence made by Hill and Solandt but which is not the simple direct one insisted upon by the Lapicques.⁵

Confirmation of the effect of electrotonus on λ is seen (1) in the observation by Parrack⁶ that accommodation at the anode is smaller than at the cathode, (2) in the decline of excitability following the initial rise during the passage of a linearly increasing current (Fabre⁷) instead of a rise in excitability to a maximum which should theoretically be maintained, and (3) in the decrease of "Einschleichzeit" (which Hill has shown is related to λ) obtained by Schriever⁸ with catelectrotonus.

11366

Occurrence of Tremors and Incoördination in Vitamin E-Deficient Adult Rats.

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Paralysis in adult rats grown and maintained on a vitamin E-deficient diet was first described in detail by Ringsted¹ and later by Burr, Brown and Moseley.² Einarson and Ringsted³ reported degenerative changes in the central nervous system and voluntary muscles, that were prevented but not cured by wheat germ oil. The

⁴ Nivet, M., *C. R. Soc. Biol.*, 1934, **116**, 1013; *Ibid.*, 1939, **131**, 262.

⁵ Lapicque, L., and M., *C. R. Soc. Biol.*, 1937, **125**, 260; *Ibid.*, 1938, **129**, 724.

⁶ Parrack, H. O., *Am. J. Physiol.*, 1939, **126**, 597; *Proc. Am. Physiol. Soc.*, 52nd Annual Meeting, 1940, p. 142.

⁷ Fabre, P., *C. R. Soc. Biol.*, 1934, **116**, 1065.

⁸ Schriever, H., *Zeitschr. f. Biol.*, 1932, **93**, 123.

¹ Ringsted, A., *Biochem. J.*, 1935, **29**, 788.

² Burr, G. O., Brown, W. R., and Moseley, R. L., *PROC. SOC. EXP. BIOL. AND MED.*, 1937, **36**, 780.

³ Einarson, L., and Ringsted, A., 1938, *Effect of Chronic Vitamin E Deficiency on the Nervous System and the Skeletal Musculature in Adult Rats*, Oxford University Press.

muscle pathology has also been described by Evans, Emerson and Telford.⁴ We have previously reported⁵ the prevention of gross symptoms with a vitamin E concentrate, and Knowlton, Hines and Brinkhous⁶ have demonstrated that alpha-tocopherol acetate will prevent or cure the muscle changes occurring prior to the appearance of gross symptoms.

This paper concerns the production of paralysis within 8 to 10 months in rats obtained from a stock receiving ample amounts of vitamin E, the symptomatology of these animals, and their response to a vitamin E concentrate.

Four male and 4 female rats weighing 35 g were placed on a highly purified vitamin E-deficient diet containing but 0.0056% of non-vitamin lipids.⁵ One female died at 15 weeks and one at 29 weeks of unknown causes. At 32 to 40 weeks the remaining animals developed the first stage of paralysis as manifested by a spreading of the hind legs and a marked lowering of the posterior abdominal region while walking. The weekly administration of 40 mg of a vitamin E concentrate,⁷ possessing antisterility activity in a single 3 mg dose, to one of the female rats showing the first signs of paralysis prevented the development of further symptoms during the remaining 37 weeks of the experiment.

By 45 to 50 weeks the untreated animals showed the second stage of paralysis characterized by extreme abduction of the hind legs, which were now practically useless for locomotion. In another 10 to 12 weeks the disease had become so severe that in walking the hind quarters were dragged along the floor with both legs swinging from side to side. This, the third stage of paralysis, had not developed in the untreated female by the 73rd week of the experiment.

Several weeks after the second stage of paralysis the untreated animals, particularly the males, developed tremors and incoördination of the forelegs and head. So severe did these symptoms become that when eating, the rats were unable to maintain their fore feet in one position in the low food pans. The continuous jerking of the head rendered unsuccessful many of the attempts to obtain a mouthful of food. The tremors, which were most marked when the

⁴ Evans, H. M., Emerson, G. A., and Telford, I. R., *Proc. Soc. Exp. Biol. and Med.*, 1938, **38**, 625.

⁵ Mackenzie, C. G., Mackenzie, J. B., and McCollum, E. V., *Biochem. J.*, 1939, **33**, 935.

⁶ Knowlton, G. C., Hines, H. M., and Brinkhous, K. M., *Proc. Soc. Exp. Biol. and Med.*, 1939, **42**, 804.

⁷ Mackenzie, C. G., Mackenzie, J. B., and McCollum, E. V., *Pub. Health Rep.*, U. S. P. H. S., 1938, **53**, 1779.

animals moved, were intensified by a shrill note from an air hose. This stimulus caused 3 of the animals with stage 2 paralysis to run frantically around the cage for 20 to 30 seconds. The rear legs were used during this remarkable outburst of activity which terminated in collapse without convulsions or loss of consciousness.

From 3 to 10 weeks after the appearance of the first stage of paralysis the weights of the male rats (330 to 380 g) began to decline. At the end of 16 to 20 weeks they had lost 60 to 100 g. A representative weight curve is shown in Fig. 1.

Attempts to cure the paralysis in 2 of the males through the administration of 40 mg of the vitamin E concentrate per week for 16 and 20 week periods were unsuccessful. However, the progress of the neuro-muscular symptoms was definitely arrested and a growth response was elicited (Fig. 1). Doubling the carotene or methyl linolate intake or supplementing with 0.6 g of ether-extracted yeast daily failed to retard the development of symptoms or the decline in weight.

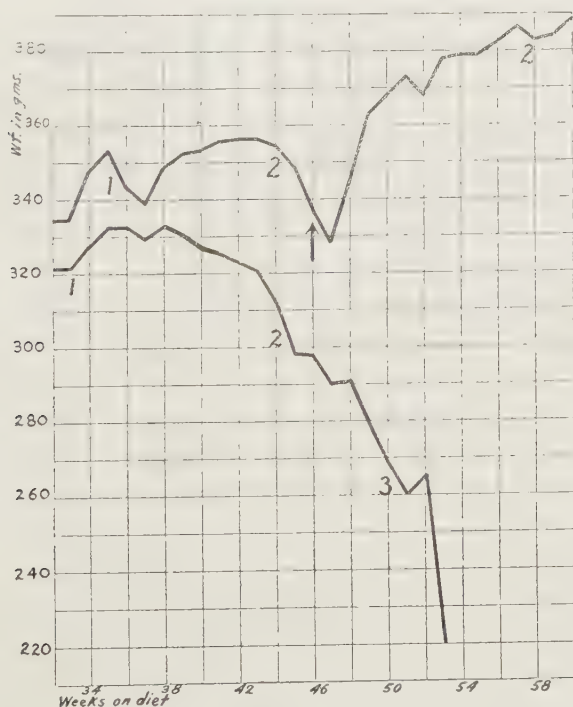


FIG. 1.

Weight curves of adult male rats reared and maintained on a low-fat vitamin E-deficient diet. Numerals indicate stage of paralysis. \uparrow indicates the addition to the diet of a vitamin E concentrate.

Microscopic examination of the thigh muscles revealed lesions similar to those described by other workers.^{3, 4, 6} These lesions were not so extensive as those occurring in young rabbits with severe nutritional muscular dystrophy, a condition cured by vitamin E.⁸

Summary. Rats grown and maintained on a highly purified vitamin E-deficient diet developed paralysis of the rear legs accompanied by tremors and incoördination of the fore legs and head. Although cures could not be obtained, the administration of a vitamin E concentrate arrested the development of these symptoms and stimulated growth.

11367 P

A Test Proposed to Measure Vitamin B₁ Saturation in Humans.

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A test designed to measure the individual patient's saturation with respect to Vitamin B₁ would have a wide clinical application. Experiments have been carried out in an attempt to develop such a procedure. The validity of such a test depends upon, among other things, the accuracy of the method of assay for the Vitamin B₁. The assay methods fall into two categories—chemical and biological. The chemical methods on the whole are specifically for pure thiamin. Among the biological methods the Schultz, Atkins and Fry technic which employs the rate of fermentation of glucose by a yeast is the most suitable for clinical investigation. This method measures not only the thiamin but the pyrimidines as well. The pyrimidines present in the urine may be considered for practical purposes as originating from the members of the B complex. The actual test in its present form employed in this laboratory is summarized as follows:

Patients were injected with 1 mg of thiamin hydrochloride intramuscularly in the fasting state. The urine was collected for a 4-hour period following the injection. The Vitamin B₁ activity of this collected urine was assayed by means of the Schultz, Atkins and Fry yeast fermentation method. Fig. 1 shows the tabulation of the

⁸ Mackenzie, C. G., and McCollum, E. V., *J. Nutr.*, 1940, **19**, 345.

B₁ Tolerance in 349 Miscellaneous Patients

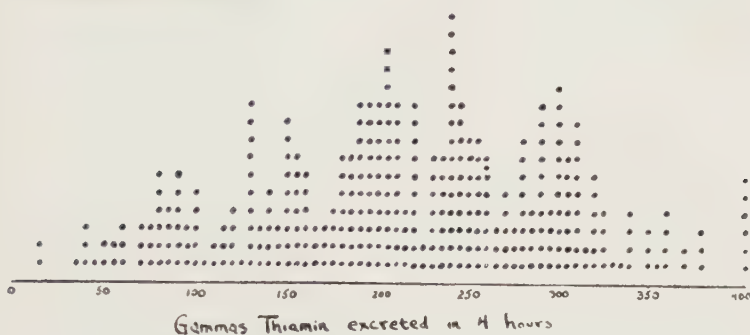


FIG. 1.

results of the test on 349 unselected patients. The distribution curve of the results indicates that an excretion of 180 gammas or more in the 4-hour period represents the average normal. Patients excreting less than 180 gammas may be considered as below average saturation.

This group of patients represents the population of hospitals and dispensaries and cannot be considered a representative cross section of the population. Fig. 2 shows the tolerance test on 35 normal, healthy students, instructors and physicians. Here it is seen that excretion values range higher. On the same figure are shown values of selected groups of patients suffering with gastro-intestinal diseases and another group in cardiac failure. The differences in range of excretion are more than significant.

Because of the known relation between carbohydrate metabolism and Vitamin B₁ a group of 132 patients with diabetes mellitus were subjected to the test. The distribution curve of the excretion values

35 NORMAL INDIVIDUALS

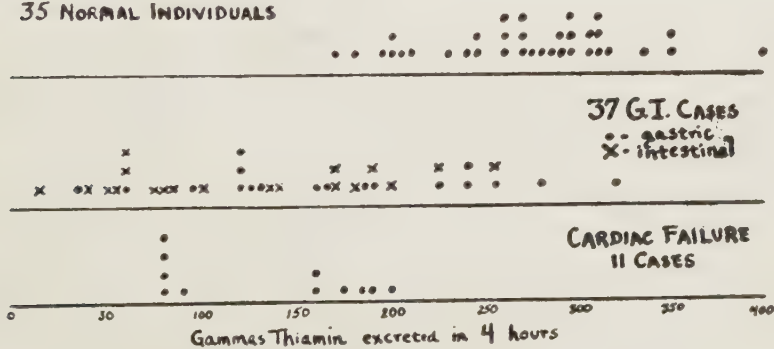


FIG. 2.

Diabetes Mellitus

132 patients

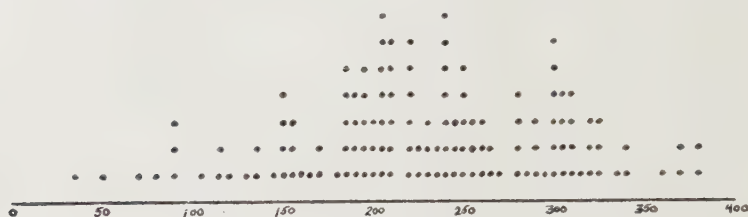


FIG. 3.

so closely parallels that of the larger unselected group of patients that one can infer no particular unsaturation of Vitamin B₁ in clinical diabetes mellitus.

11368

Menstrual Discharge of Women. I. Its Toxicity in Rats.*

O. WATKINS SMITH AND GEORGE VAN S. SMITH.

From the Fearing Research Laboratory, Free Hospital for Women, Brookline, Mass.

In an effort to find a factor to which the local processes resulting in menstruation might be attributable, 37 specimens of menstrual discharge, each varying from 30 to 120 cc in amount, donated by 5 normally menstruating, parous women, have been studied. They have been collected by means of soft rubber cups.† During collection each portion has been placed in the refrigerator immediately upon removal. For the most part, the experiments to be reported have been performed upon the whole specimen after pooling the various portions. For control experiments, whole venous blood, drawn during the first day of menstruation and citrated or mixed with sufficient distilled water to prevent clotting and kept in the refrigerator, has been used in similar or larger amounts.

Menstrual discharge has been found to be highly toxic to rats, their resistance being markedly affected by hormonal conditions.

* The Mrs. William Lowell Putnam Investigation of the Toxemias of Pregnancy, aided by grants from the Committee for Research in Problems of Sex, National Research Council.

† The Hy-Kup Distributors (National), Indianapolis, Indiana.

Toxicity varies with different specimens and is more concentrated in the cells and debris than in the "plasma." Whole menstrual discharge given subcutaneously in 0.1 to 1.0 cc amounts (depending upon the specimen) twice daily to normal mature females, starting when they are in preëstrus, often results in death within 48 hours. Within 24 hours or less of the first injection the animals are apprehensive and "hunched up"; their fur is ruffled; water intake increases; the nose and inner canthi of the eyes become encrusted with blood and there is firm edema of a wide area around the site of injection. Death is not ushered in by convulsions. At autopsy, the adrenals are of a dark reddish-brown color, the liver is usually dark and mottled and the lungs are congested, as compared with controls. Occasionally blood is found in the urinary bladder. Under the microscope the lungs show edema and capillary hemorrhage, the kidneys varying degrees of parenchymatous degeneration, congestion and often capillary hemorrhage. The liver shows some degeneration. The most consistent picture is seen in the adrenal cortex, diffuse or focal hemorrhage and dissolution of cells in either or all zones. In the adrenal cortices of rats that have died early, hemorrhage may be the only finding. With increased length of survival, dissolution to actual necrosis of cells is also seen, along with increased vacuolization.

Animals in which injections are started during the beginning of postestrus are most likely to survive and become comparatively resistant to continued injections. On exploration after the third or fourth day, their ovaries contain large, red corpora lutea. The subcutaneous edema becomes replaced by brawny induration, so that in less than 15 days the stiffness and fixation of the pelt prohibit further injections.

If each dose of menstrual discharge is accompanied by the subcutaneous injection of 1 to 2 r.u. of a native estrogen (we usually have used 0.05 γ of estradiol), the animals almost invariably die within 48 hours of the first injections, regardless of the time of the cycle at which administration is started. Clear "plasma" from the discharge is usually innocuous in normal mature female rats in fairly large amounts (1 to 2 cc twice daily) at any stage of the cycle unless estrogen is concomitantly given, in which case the typical rapid death ensues. In control experiments with venous blood or serum with or without estrogen, the only deleterious effect noted was subcutaneous induration when more than 2 cc of whole venous blood was administered daily.

The most consistently lethal effect has been noted in 19- to 24-

day-old female rats. Thirty-five of the 37 specimens so tested have produced death, when given twice daily, in less than 48 hours from the first injection, the total dose varying with different specimens between 0.01 and 0.8 cc. The only 2 specimens non-lethal in 0.8-cc amounts in immature females were from the same donor and had been collected during the first day of the period when flow was profuse and contained little or no debris. In this individual, specimens during the last days of flow contained much brown debris and were very much more toxic, a single injection of 0.01 to 0.1 cc being usually lethal to immature female rats. Two to 4 cc of whole venous blood in 4 doses over 2 days is easily tolerated by immature female rats. Spayed immature female rats, and male rats, both immature, mature and castrated mature, have a relatively greater resistance to the discharge, and mature females, spayed more than 4 weeks previously, have tolerated even larger amounts. The administration of estrogen to spayed females does not render them more susceptible to the discharge. In 2 experiments, a mash of 6 to 7 fresh mature rat ovaries injected simultaneously with each dose of the discharge and estrogen produced the typical rapid death in spayed females; the control spayed females receiving the same amounts of the same discharge and estrogen survived.

From these observations, it would appear that the greatest susceptibility to the menstrual toxin depends upon the presence of the ovaries and that corpora lutea afford partial protection, but that the administration of estrogen overrides this protective action.

The toxicity of the discharge is destroyed by heat, ethyl alcohol, acetone or acid, in amounts sufficient to precipitate the proteins. It is diminished by raising the pH to over 8 or by allowing the specimens to become putrid either by standing at room temperature for a few days or in the refrigerator for at least 3 weeks. Dried rapidly *in vacuo* over CaCl_2 , powdered, sealed and refrigerated, the material retains its toxicity for at least 8 months. The toxin is not soluble in the usual lipid solvents and is nondialysable. After fractional precipitation with $(\text{NH}_4)_2\text{SO}_4$, it is found in greatest concentration in the water insoluble portion (after dialysis) of the englobulin precipitate.

Mature female rats have been completely protected against a lethal dose of menstrual discharge plus estrogen by the preliminary and simultaneous administration of large amounts of progesterone, to a total of 15 to 30 mg. Chorionic gonadotropin, if given so as to produce a good luteinizing response by the time a M.L.D. of the discharge is injected, protects immature female rats, but not if

estrogen be given with the discharge. Chorionic gonadotropin does not save mature females from the combined injection of discharge and estrogen. Desoxycorticosterone acetate (10 to 20 mg) does not prevent death from a M.L.D. of discharge in immature females or from discharge plus estrogen in mature females even when its administration is begun 2 days before injecting the toxin. Adrenal cortical extract (Eschatin, P. D. & Co.) in 1-cc doses twice daily for 2 days before and during the day of injection of twice the M.L.D. of discharge completely protected an immature female; a littermate control receiving the same amount of the same discharge on the same day died in 20 hours.

Immature and mature female rats may be rendered resistant to several times the M.L.D. of discharge alone or discharge plus estrogen by injection of sublethal doses twice a week for 2 to 3 weeks. Such immunity has been maintained for as long as 4 months. Mature female rabbits are extremely susceptible to the discharge alone, 1 to 3 subcutaneous injections of 1 cc usually resulting in death within 48 hours. By extreme caution, 1 rabbit was made to survive 14 days of small injections. Its serum, in 5 1-cc amounts over 2½ days prior to the injection of twice the M.L.D. of a specimen, completely protected an immature female rat. A pseudoglobulin fraction of this same serum also protected immature female rats against lethal doses of 2 other samples of discharge from 2 different donors.

Only by preliminary treatment were we able to protect rats against the menstrual toxin with progesterone, adrenal cortical extract or immune serum. After the discharge had been administered, even massive amounts of any of these failed to prolong the period of survival.

In an attempt to determine whether the toxicity of menstrual discharge is due to a specific toxin or simply to products of bacterial action or protein decomposition, the following experiments were run. Sterile citrated whole venous blood was incubated for 48 hours after inoculation with discharge. This material was toxic to immature rats but not lethal, even in amounts 3 to 5 times the M.L.D. of fresh discharge. The striking edema which characterizes the reaction to menstrual discharge was lacking in rats injected with inoculated venous blood. Furthermore, it was found that sterile venous blood alone, after 48 hours at incubation temperature, is as toxic as inoculated material. These experiments, therefore, gave no conclusive evidence either for or against the specificity of menstrual toxin. Discharge collected on the fourth day of flow in a sterile cup following a douche with 2 quarts of water killed an

immature female rat in 30 hours. This material was given in a single injection of 0.25 cc within 40 minutes from the time it passed the cervix. Its toxicity would seem to rule out bacterial activity or any protein decomposition other than what might occur in the uterus. The strongest indication of a specific toxin lies in the repeatedly confirmed observation that 1 to 2 cc twice daily of specimens of whole discharge or "plasma," which alone in these amounts are non-toxic to normal mature female rats, are lethal within 72 hours from the first injection when estrogen is simultaneously administered, whereas such is not the case with venous whole blood or serum.

Conclusion. The menstrual discharge of women with normal cycles is highly toxic to rats through the production of vascular damage. The possibility that this toxicity is accountable to protein decomposition has not been conclusively ruled out, although the marked effect of hormonal conditions upon resistance appears to argue against this. The greatest susceptibility requires the presence of the ovaries. Functional corpora lutea afford partial protection, but the administration of estrogen overrides this action. Protection may be rendered by pretreatment with large amounts of progesterone, adrenal cortical extract or "immune" rabbit serum. The toxin appears to be intimately associated with a large moleculued protein material.

11369 P

Menstrual Discharge of Women. II. Its Progesterone-Stimulating Effect in Mature Rats.*

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From the Fearing Research Laboratory, Free Hospital for Women, Brookline, Mass.

Early in the study of the toxicity of the catamenial discharge¹ it was noted that mature female rats, with previously regular cycles, which survived the first 2 or 3 days of injections went, within 72 hours, into constant diestrus on continued injections and reverted

* The Mrs. William Lowell Putnam Investigation of the Toxemias of Pregnancy, aided by grants from the Committee for Research in Problems of Sex, National Research Council.

¹ Smith, O. W., and Smith, G. V., *Proc. Soc. Exp. Biol. and Med.*, 1940, **44**, 100.

to normal cycles when injections were discontinued. One to 2 cc twice daily of either whole menstrual discharge or "plasma" produce this effect, but "plasma" (without estrogen) is less likely to be lethal and is not as irritating to the subcutaneous tissues. Exploration revealed large corpora lutea which were functional, as demonstrated by the finding of deciduomata on the ninth day of injections following needling of the uterus on the sixth day.

Such a progesterone-stimulating action suggested that the discharge might contain either a known gonadotropic hormone or an estrogen, although the familiar estrogens in amounts sufficient to stimulate demonstrable hyperactivity of the corpora would first produce estrous vaginal smears. In searching for estrogenic potency, we have extracted large amounts of whole menstrual discharge, "plasma" and dried whole discharge with ethyl alcohol, ether, acetone, butyl alcohol and benzene. We have also attempted to recover any combined estrogen by performing acid hydrolysis upon wet and dried whole discharge and butyl extracts of the same.† None of these preparations, in olive oil solution or saline suspension, has given estrus in spayed mature female rats, standardized for estrogen assay, even when tested upon a number of primed animals for as little as 1 r.u. in 20 cc of material.‡ Although Frank³ has reported estrogenic activity in menstrual discharge, our results demonstrate that, if present at all, there is not enough to account for the increased production of progesterone in normal mature rats. These lipid extracts have neither been toxic nor had any effect upon the cycles of rats.

In searching for gonadotropic substance, whole discharge was shaken with 5 volumes of 95% ethyl alcohol and placed in the refrigerator overnight. After centrifugation, the precipitate was washed twice with ether, dried, powdered and taken up in water. After 24 hours in the refrigerator, with repeated shaking, the water-

† Crystalline estrone in water is rapidly destroyed by acid hydrolysis methods which may be used upon urine without loss of added estrone. A solution of high salt concentration (69 g NaH_2PO_4 and 179 g Na_2HPO_4 per L) has been found a satisfactory substitute for urine and used as the medium for hydrolysis in attempting to deconjugate any combined estrogens in menstrual discharge or extracts of it. The material to be tested has been diluted with 10 volumes of concentrated phosphate solution, boiled under a reflux for 10 minutes with 15 vol.% HCl^2 and extracted for 24 hours in a continuous benzene extractor.

² Smith, O. W., Smith, G. V., and Schiller, S., *Endocrinol.*, 1939, **25**, 509.

‡ A total of 22 lipoidal extracts of whole menstrual discharge or plasma have been tested, each representing from 40 to 270 cc of material.

³ Frank, R., *The Female Sex Hormones*, Charles C. Thomas, published 1929.

insoluble residue was separated by centrifugation, washed 3 times with water and discarded, the watery extract (with washings) being made up to a measured volume and used for testing. Such extraction appears to yield a complete recovery of the progesterone-stimulating principle, since, with 12 specimens from 3 donors, the equivalent of 1 cc of discharge twice daily for 8 days was sufficient to increase and prolong the activity of corpora lutea in mature rats, with the production of deciduomata after needling of the uterus on the sixth day. These extracts have shown no toxicity other than a slight subcutaneous reaction. As tested on spayed rats, they contained no free or combined estrogen.† They have had no effect upon the genitalia of immature female rats in amounts equivalent to 6 cc of whole discharge. The combined injection of one of them and F.S.H. (from menopausal urine) into an immature female rat gave no luteinization. They have not enhanced the luteal response of immature rats to chorionic gonadotropin and have repeatedly failed to augment the weight of the seminal vesicles of immature males (the equivalent of 10 cc of material being given in 10 doses over 5 days). These findings rule out the presence of any known follicle-stimulating or luteinizing hormone. Furthermore, activity was not diminished by heating in a boiling water bath for 1 hour, which treatment is destructive of the known gonadotropic hormones.

After fractionation of the proteins of menstrual discharge with $(\text{NH}_4)_2\text{SO}_4$, the progesterone-stimulating activity is recovered in the water-insoluble portion (after dialysis) of the englobulin precipitate. The toxicity of menstrual discharge is also recovered in greatest concentration in this fraction.§ The toxin and progesterone-stimulating substance are not identical, however, since alcohol precipitation destroys the former but not the latter. Furthermore, the latter has been found in nonlethal materials, such as alcohol-ether precipitates of venous blood and urine at the time of menstruation and in a sample of catamenial discharge that had lost its toxicity on standing. None has been demonstrable in venous blood, in the amounts tested, during the luteal phase of the cycle or in blood from males. In testing for it, upon normal mature rats with previously regular cycles, the result is considered positive only when deciduomata, confirmed by microscopic section, are present on the eighth or ninth day of injections after needling of the uterus on the fifth or sixth day.

§ These findings suggest that the progesterone-stimulating factor occurs in menstrual discharge in a toxic protein conjugation which is split by alcohol but not by $(\text{NH}_4)_2\text{SO}_4$ precipitation.

Conclusion. The menstrual discharge of normally menstruating women contains a heat-stable factor, insoluble in lipoid solvents, which stimulates increased luteal activity in mature rats. This factor has also been found in venous blood and urine at the time of menstruation. Its progesterone-stimulating effect is not accountable to any free or combined estrogen or to any known gonadotropic hormone.

So far as this work has progressed, the progesterone-stimulating factor of menstrual discharge resembles the substance reported by Astwood and Greep as occurring in the rat placenta.⁴ Our material has not yet been tested upon hypophysectomized rats but the fact that alcohol precipitation destroys toxicity without decreasing progesterone-stimulating activity makes it appear that the progesterone stimulation is not a non-specific toxic effect.

11370

Unsuccessful Therapy in Experimental Equine Encephalomyelitis with Salt Solutions of Varied Concentrations and Sulfanilamide Compounds.*

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The high mortality of Eastern equine encephalomyelitis in both man and animals has prompted interest in therapy. Specific antisera may have value in the treatment of horses if administered early, but its use after the disease is well established is ineffectual.¹ In horses the disease may be suspected and treated specifically; however, in man the diagnosis must usually await the appearance of neurological signs at which stage the lesions are too far advanced for antiserum therapy. This shortcoming suggested the investigation of other therapeutic procedures employing the highly susceptible rat and mouse.

The beneficial effect of hypertonic solutions, such as 10-25%

⁴ Astwood, E. B., and Greep, R. O., *PROC. SOC. EXP. BIOL. AND MED.*, 1938, **38**, 713.

* This work was aided by a grant from the Clara Ward Seabury Clinic for Infantile Paralysis.

¹ Personal communication, Dr. B. M. Lyon, Assistant Director, Vet. Dept., Lederle Labs., New York.

glucose, in edema of the CNS has long been recognized, particularly in edema of traumatic origin. In contrast, poliomyelitis of man and monkey has apparently responded favorably to injections of hypotonic solutions in the hands of Retan.² Therefore, the effects of hypertonic and hypotonic saline solutions were compared in rats with experimental EEE using isotonic saline as a control. Intra-abdominal injections of the solutions were begun 40 hours after intracranial inoculation of the rats, at which time about 10% of the animals showed well advanced signs of the disease. The remaining rats appeared normal. The fluids were administered in 4 cc quantities every 2 or 3 hours, totaling approximately 40 cc daily for a 150 g rat. This is equivalent to 12 liters for a 60 kg man. The saline injections were continued until 83% of the animals were dead. This treatment in no way altered the course of the infection (Table I).

Many workers have reported the use of sulfanilamide and related compounds on virus infections of man and animals. In man sulfanilamides seem to be of value in lymphopathia venereum,³ and in animals success has followed the treatment of meningo-encephalitis associated with canine distemper,⁴ with negative results in poliomyelitis,^{5, 6, 7} rabbit myxoma, rabbit fibroma, herpetic encephalitis, choriomeningitis and St. Louis encephalitis.⁸

The following compounds were selected for evaluation in the treatment of EEE in rats and mice: sulfanilamide, sulfapyridine,

TABLE I.
Effect of Intraabdominal Injections of Saline Solutions of Varied Concentrations on EEE in Rats Infected with 10 MCLD.*†

Treatment			No. of rats	Mortality
Solution	Dose, cc	Frequency		
None (control)			45	36 (80)
Hypertonic Saline—3%	4	Every 2-3 hr	45	39 (86)
Hypotonic Saline—0.375%	4	" "	45	39 (86)
Isotonic Saline—0.875%	4	" "	45	36 (80)

*Duration of the experiment was 72 hr.

†MCLD = Minimal cerebral lethal doses.

2 Retan, G. M., *J. Ped.*, 1937, **11**, 647.

3 Shaffer, L. W., and Arnold, E., *Arch. Derm. and Syph.*, 1938, **38**, 705.

4 Marcus, P. M., and Necheles, H., *Proc. Soc. Exp. Biol. and Med.*, 1938, **38**, 385.

5 Toomey, J. A., and Takacs, W. S., *Arch. Ped.*, 1938, **55**, 307.

6 Kelsen, S. R., *Proc. Soc. Exp. Biol. and Med.*, 1937, **36**, 718.

7 Toomey, J. A., and Takacs, W. S., *Arch. Ped.*, 1939, **56**, 384.

8 McKinley, E. B., Meek, J. S., and Acree, E. G., *J. Infect. Dis.*, 1939, **64**, 36.

2-sulfanilamidothiazol (sulfathiazol), 2-sulfanilamidomethylthiazol (sulfamethylthiazol), and 2-sulfanilamidophenylthiazol (sulfaphenylthiazol). The animals were inoculated intracerebrally. Treatment with the drugs was started 24 hours later and was continued twice daily by the intraabdominal route. The rats received 40 mg of sulfanilamide (human dose of sulfanilamide compounds: maximum 6 g daily *per os*); 2 mg of sulfapyridine, and 15 mg of the thiazol compounds. The 5 sulfanilamide compounds were injected into the mice in 2 mg doses following the aforementioned technic. This procedure is open to criticism based on the clinical opinion that in order to maintain a satisfactory blood level of the drug it must be given 4 times daily. However, in the hands of Barlow and Homburger⁹ the treatment of staphylococcus infections of mice with 2 daily doses of thiazol compounds administered by stomach tube was successful. In addition, 1,000 times the minimal cerebral lethal dose may be considered a too severe inoculum, but in man the virus would be present in high con-

TABLE II.
Chemotherapy of EEE in Rats Infected with 1,000 MCLD.*

Treatment		No. of rats	Mortality %
Compound	Dose, mg twice daily		
None (control)		16	16 (100)
Sulfanilamide	40	14	14 (100)
Sulfapyridine	2	14	14 (100)
Sulfathiazol	15	12	12 (100)
Sulfamethylthiazol	15	9	8 (89)
Sulfaphenylthiazol	15	9	9 (100)

*See footnote Table I.

TABLE III.
Chemotherapy of EEE in Mice Infected with 1,000 MCLD.*

Treatment		No. of rats	Mortality %
Compound	Dose, mg twice daily		
None (control)		12	12 (100)
Sulfanilamide	2	12	12 (100)
Sulfapyridine	2	12	11 (92)
Sulfathiazol	2	12	11 (92)
Sulfamethylthiazol	2	12	10 (83)
Sulfaphenylthiazol	2	12	12 (100)

*See footnote Table I.

⁹ Barlow, O. W., and Homburger, E., *PROC. SOC. EXP. BIOL. AND MED.*, 1939, **42**, 792.

centration before the diagnosis could be made and the drug administered.

The data are presented in Tables II and III. The drugs under the conditions of the experiment had no apparent effect on the course of the infection.

11371 P

Growth Promotion of the Tubercle Bacillus by Serum Albumen.

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The author¹ described the growth of single colonies of tubercle bacilli in the depth of coagulated rabbit plasma. It was then observed that rabbit serum + agar was a less favorable medium than coagulated plasma. Only a few colonies developed in "hormone agar", but many more in hormone agar + rabbit serum. The growth-promoting effect of serum was quite variable. Evans and Hanks² confirmed the favorable effect of rabbit serum and obtained good growth in the depth of Long's medium after the addition of blood or serum. Kallós and Nathan³ and Pagel⁴ found that some human sera support the growth of tubercle bacilli while others fail to do so. Pagel could not demonstrate the presence of either growth-promoting or specific inhibiting substances. Drea⁵ confirmed the inhibiting effect of agar and found that tubercle bacilli grew in the depth of a modified Long's medium when it had been inoculated with varying quantities of a bacillary emulsion.

The addition of human, guinea pig, rabbit, sheep or horse serum all enhance the growth of tubercle bacilli in the depth of synthetic medium. The growth appears earlier, is more abundant and takes place after inoculation of smaller quantities. In synthetic medium where the nitrogen is supplied by glycine, growth rarely occurs after inoculation of less than 10^{-1} mg tubercle bacilli, in media with asparagine-ammonium citrate as nitrogen source, growth frequently

¹ Boissevain, C. H., *Am. Rev. Tuberculosis*, 1926, **13**, 90.

² Evans, B., and Hanks, J. H., *Proc. Soc. Exp. Biol. and Med.*, 1939, **40**, 112.

³ Kallós, P., and Nathan, E., *Z. f. Immunitäts forschung*, 1932, **76**, 393.

⁴ Pagel, W., *Tubercle*, 1934-35, **16**, 256; *J. Path. and Bact.*, 1940, **50**, 111.

⁵ Drea, W. F., *J. Bact.*, 1940, **39**, 197.

occurs after inoculation of 10^{-3} mg tubercle bacilli, but either medium will produce profuse growth after inoculation of 10^{-6} mg bacilli if 5% unheated serum is added to the medium.

The total lipoids extracted from the serum by alcohol and ether are inhibiting and the phospho-lipins are without effect in contrast to what was observed in egg yolk.⁶ When the proteins are precipitated by alcohol and redissolved in distilled water, they have the same growth-promoting effect as the original serum, while the filtrate (after removal of the alcohol) is inhibiting.

Separation of the serum proteins in albumen and globulin by half saturation with ammonium-sulfate, showed the albumen fraction to be strongly growth-promoting (after dialysis), while the globulin fraction was inhibiting or without effect. Crystalline horse serum albumen after three recrystallizations was strongly growth-promoting when added in a concentration of 0.1% to synthetic medium; rapid and abundant growth occurred after inoculation of as little as 10^{-7} mg tubercle bacilli. Autoclaving for 10 minutes at 115°C of the synthetic medium containing the serum albumen destroys the growth-promoting effect.

Horse serum albumen after 3 recrystallizations still contains numerous impurities, amongst which are globulins, enzymes, pigments and flavoprotein. Experiments are now in progress to prepare a horse serum albumen completely free of such admixtures.

The growth-promoting activity of crystalline horse serum albumen is much greater than that of the lipid extracts of egg yolk previously described. Addition of 0.3% egg yolk gave good growth

TABLE I.
No. of Colonies of Tubercle Bacilli Developing in Depth of Media After Inoculation of Varying Amounts of Bacilli.

Mg tubercle bacilli inoculated	Glycine medium	Asparagine-ammonium citrate medium	Glycine medium with 0.1% crystalline horse serum albumen	Asparagine-ammonium citrate medium with 0.1% crystalline horse serum albumen
10-1	7	innumerable	innumerable	innumerable
10-2	0	8	"	"
10-3	0	3	"	"
10-4	0	0	"	"
10-5	0	0	"	"
10-6	0	0	80	"
10-7	0	0	6	30
10-8	0	0	0	1

⁶ Boissevain, C. H., and Schultz, H. W., *Am. Rev. Tuberculosis*, 1938, **38**, 624.

after planting 10^{-5} mg bacilli while 0.1% horse serum albumen supports growth after planting 10^{-7} mg bacilli.

Summary. Crystalline horse serum albumen was shown to be strongly growth-promoting for tubercle bacilli grown in synthetic media. Horse serum globulins were without effect or inhibited growth.

11372 P

Association of Tetanospasmin with Hemoglobin in Acute Stages of Tetanus Intoxication of Guinea Pigs.

GREGORY SHWARTZMAN.

From the Laboratories of The Mount Sinai Hospital, New York, N. Y.

The object of this communication is to report on a new observation that tetanus toxin (tetanospasmin) may be found intimately associated with the hemoglobin of guinea pigs during the paralytic stages of the disease. A part of an extensive series of experiments on this problem is briefly summarized, as follows:

Guinea pigs weighing each 350 g were injected subcutaneously in the abdominal region with tetanus toxin* in doses ranging between 20 to 40 M.L.D. and bled from the heart during the stages of complete paralysis and spasmodic contractions (*i.e.*, 24-48 hours following the subcutaneous injection). The pooled blood was defibrinated by shaking with glass beads; filtered through several layers of gauze, and centrifuged at room temperature for one-half hour. The clear, dark red supernatant plasma and the sedimented red blood cells were placed in separate containers.

Washing of red blood cells in 1% NaCl solution was repeated until the supernatant fluid became biuret-negative (usually 4 washings are required). The washed cells were diluted 1:5 in distilled water. When fairly complete laking and some crystallization occurred about one hour later, the solution was centrifuged for one-half hour also at room temperature. This procedure resulted in separation of a dense dark red sediment containing masses of hemoglobin crystals; a superimposed loose whitish precipitate consisting of cellular debris and some hemoglobin crystals; and finally, a clear dark red supernatant fluid.

* Tetanus toxin in powder form obtained from Eli Lilly Research Laboratories (Lilly 27994, 400,000 M.L.D. per g), through the courtesy of Dr. H. M. Powell.

The serum was fractionated with the aid of the somewhat modified method of Parsons,¹ originally recommended for crystallization of hemoglobins and in these experiments consisting of repeated alternate freezing to -14°C and centrifugalization of the serum at 4°C until complete thawing. The procedure enabled the separation of colorless, faintly colored, medium colored, and dark red fractions. Small amounts of crystals and whitish debris were also obtained.

Following determinations of the concentration of hemoglobin by the Sahli method modified by Wintrobe (Hellige haemometer), all the materials were promptly injected into guinea pigs weighing each 350 g. The volume injected into each guinea pig ranged between 1 cc-10 cc and never exceeded 10 cc. One cc was given subcutaneously over the abdomen, the remaining amount was introduced subcutaneously over both thighs in equally divided doses. Examinations for symptoms of generalized and local tetanus were made daily. Test guinea pigs recorded as having shown tetanus intoxication died following generalized paralysis and spasms. On post-mortem examination no other pathological findings were observed except localized inflammation at the site of injection without pus formation, and some congestion of lungs and adrenals. The results of the tests were consistently as follows:

The blood defibrinated by means of glass beads elicited tetanus.

The washed red blood cells, the cellular debris and packed hemoglobin crystals suspended in a small amount of 0.85% NaCl solution failed to produce the symptoms.

Laked red blood cells tested before centrifugalization, and the clear dark red supernatant fluid obtained after centrifugalization of the laked cells, both possessed a considerable amount of tetanus toxin, which in sufficient concentration produced acute paralysis and death within 48 hours following the subcutaneous injection.

The plasma containing a certain amount of hemoglobin also had the tetanospasmin. Fractionation proved, however, that the major portion of the toxin was associated with the dark red portion; a small amount capable of producing only local tetanus being present in the medium-colored fraction, and none at all found in the colorless and slightly colored parts.

Quantitative studies emphasize the remarkable fact that under the above experimental conditions the tetanospasmin concentration of the blood is approximately directly proportional to the hemoglobin concentration. With doses 20-40 M.L.D. of the toxin injected sub-

¹ Parsons, quoted in *The Respiratory Function of the Blood... Part II. Hemoglobin*, p. 68, by Joseph Barcroft, Cambridge, University Press, 1928.

cutaneously per guinea pig, weighing 350 g, each M.L.D. recovered from the blood of the intoxicated guinea pigs corresponded to 0.035 g-0.007 g of hemoglobin in the preparations and fractions tested, in volumes ranging between 1 cc-10 cc. The effects of these total amounts of hemoglobin were the same, irrespective of the volumes used.

The active blood preparations may be considered predominantly oxyhemoglobin. Occasional batches of methemoglobin obtained after prolonged storage and reduced hemoglobin were not studied in this series.

No symptoms were elicited in test-guinea pigs with blood fractions of control guinea pigs receiving no injections and of those injected with tetanus toxin heated at 80°C for 1/2 hour prior to the injection. Also, blood fractions of guinea pigs which received an old inactive toxin gave no tetanus.

In this and the following paper the term "hemoglobin" refers to complex substances obtained in solution following laking of washed erythrocytes and removal of cellular debris. Hemoglobin is defined, therefore, by the biological properties, its chemical relationships being disregarded for the moment. The term "association" is merely descriptive and indicates a combination which may either occur directly with the hemoglobin or through the intermediary of some unidentified constituent with which the hemoglobin is associated.

All the data considered together, especially observations on the toxic activity of hemoglobin obtained by means of lysis of washed red blood cells, indicate clearly that under the experimental conditions described, a close association exists between hemoglobin and tetanospasmin in the blood recovered from guinea pigs during the acute stages of the disease.

Investigations on association of certain viruses with hemoglobin are under progress.

11373 P

Association of Meningococcus and *B. typhosus* Toxins with Hemoglobin *in vitro*.

GREGORY SHWARTZMAN.

From the Laboratories of The Mount Sinai Hospital, New York, N. Y.

Incidental to the observations that a close association exists between tetanospasmin and hemoglobin in the blood of guinea pigs suffering from tetanus intoxication¹ a series of experiments was carried out on combination of hemoglobin with bacterial toxins *in vitro*. The results are briefly presented in this communication.

The toxins employed were filtrates from the "agar washings" of cultures of meningococcus and *B. typhosus* which were highly potent in the elicitation of the phenomenon of local skin reactivity.² In this work they were purified by dialysis in cellophane bags No. 600 against 0.85% NaCl solution for a period of one week.³

The hemoglobin preparations were made in the following manner: All the work was done under strict precautions of sterility and each step controlled for bacterial contamination on aerobic and anaerobic media. Rabbit blood obtained from the heart was defibrinated by shaking with glass beads, filtered through several layers of gauze and centrifuged in order to separate the plasma from the erythrocytes. The erythrocytes were promptly washed in 1% NaCl solution by repeated centrifugalization until the washings became biuret-negative (from 5-7 washings being required). The packed red blood cells were kept frozen for several hours at -70°C in a mixture of cellosolve and dry ice and gradually thawed out in a mixture of ice and alcohol and in the refrigerator at 4°C overnight. The cells were diluted in distilled water to a hemoglobin concentration of 50-60% as determined in the Hellige haemometer.

Filtration of the solution through a Seitz filter resulted in effective removal of cellular debris, as ascertained by examination of spreads stained by Wright's method and hanging-drop preparations. In control experiments adjusting the pH to 5.5 by the addition of N/10 HCl gave no precipitate in the filtrates.⁴ The clear, dark red

¹ Schwartzman, G., *Proc. Soc. Exp. Biol. and Med.*, 1940, **44**, 112.

² Schwartzman, G., *Phenomenon of Local Tissue Reactivity and Its Immunological, Pathological and Clinical Significance*, Paul B. Hoeber, Inc., Medical Book Department of Harper and Brothers, New York, 1937.

³ Schwartzman, G., Morell, S., and Sobotka, H., *J. Exp. Med.*, 1937, **65**, 323.

⁴ Jorpes, E., *Biochem. J.*, 1932, **26**, 1488.

filtrates were dialyzed at 4°C for a period of several days in cellophane bags No. 600 against distilled water. The water was changed daily. The materials removed from the bags were dried *in vacuo* by the methods of Flosdorf and Mudd. They subsequently yielded perfect solution on addition of a diluent up to the initial volume, *i.e.*, distilled water, Sørensen phosphate buffer solution of pH 6.8, plain broth or blood serum.

In the experimental work about to be described an amount of dry hemoglobin representing a yield of 0.85 g of dissolved hemoglobin was mixed with various amounts of undiluted dialyzed toxins and also toxins diluted in different diluents or in normal rabbit serum. The phenomenon-producing potency of the final concentration of toxins per one cc ranged between 50-100 reacting units. The mixtures were dried *in vacuo* in the Flosdorf-Mudd apparatus. One or several days later the dry materials were dissolved in distilled water in amounts equal to the initial volumes of hemoglobin solutions or in phosphate buffer of pH 6.8 of the same or greater volumes. The solutions were then fractionated by the method of Parsons.⁵ After 7-10 repetitions of alternate freezings at -14°C and centrifugalizations at 4°C until complete thawing, fractions widely differing in hemoglobin concentration were obtained.

The materials were assayed for toxic activity by means of the phenomenon of local skin reactivity. In these tests, the rabbits were prepared by a single intradermal injection of dialyzed meningococcus of *B. typhosus* toxin (*i.e.*, the same as used for combination with hemoglobin) and 24 hours later injected intravenously with the materials tested, one or more groups of 2 rabbits serving for each material. The results of the experiments carried out in this manner were as follows:

Rabbit, cow, guinea pig and sheep hemoglobin containing no toxin and fractionated by the method of Parsons gave no reactions in prepared rabbits.

Hemoglobin held meningococcus and *B. typhosus* toxins, the activity being associated with colored fractions and totally absent from slightly colored and colorless portions. Quantitatively, toxicity was not found in hemoglobin concentrations lower than 0.012 g per one cc. The association with the hemoglobin was firm. Elution of the toxin could not be obtained either by dilution of the toxin-hemoglobin combination in 3-fold larger amounts of the initial diluent, or by the use of plain broth, phosphate buffer mixture of

⁵ Parsons, quoted in *The Respiratory Function of the Blood. Part II. Hemoglobin*, p. 68, by Joseph Barcroft, Cambridge, University Press, 1928.

pH 6.8 and normal horse serum. The same firm association of toxin with hemoglobin was obtained when toxin mixed with normal horse serum was used for the combination. Further fractionations and elutions were done under aerobic conditions. The preparations in solution appeared of bright red color of oxyhemoglobin. Occasionally, dark brown preparations of methemoglobin were encountered. They were not studied in this series of experiments. The relation of oxidation and reduction to the association described will be embodied subsequently in a publication dealing with crystallized hemoglobin preparations.

Thus, it may be concluded from the experiments cited that hemoglobin is capable of entering into association with bacterial toxins *in vitro*. The combination appears firm since elution by greater dilution in water and by dilution in other diluents, as well as normal rabbit serum, is unsuccessful.

The stable association of toxic agents *in vivo* and *in vitro* with hemoglobin suggests studies on the effects of disease-producing agents upon intimate processes of cellular physiology in which hemoglobin and similar respiratory enzymes play an important rôle.

11374 P

Regeneration of *Euplanaria Dorocephala* with Pituitary Gland Extract.*

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From the Biological Laboratories, Temple University, Philadelphia.

The literature presents few examples of effects upon invertebrate material by vertebrate hormones.^{1, 2} This preliminary paper reports the accelerated regeneration in posterior and anterior portions of *Euplanaria dorocephala* in media of beef pituitary extracts. Wulzen³ reports the effect of feeding of ox pituitary gland upon the growth and fission of *Planaria maculata*. In the present study there was no normal feeding, for the pituitary extracts were introduced in solution and the regenerating, transected flatworms decreased in size during the experiments.

* The author wishes to acknowledge indebtedness to Dr. E. J. Larson, Temple University, for his kind interest and direction.

1 Ashbel, Rivka, *Nature* (London), 1935, **135**, 343.

2 Coldwater, K. B., *J. Exp. Zool.*, 1933, **65**, 43.

3 Wulzen, Rosalind, *J. Biol. Chem.*, 1916, **25**, 625.

The animals were cut transversely midway between the eyespots and proboscis and each part placed in a separate section dish containing 10 ml of spring water. The total number of regenerating pieces in the preliminary experiments was 112. Of these, 54 were retained as controls. Extract of whole beef pituitary in various amounts was added to the spring water in the remaining dishes, each containing a regenerating animal. The pituitary extract was prepared as follows: One gram of desiccated whole beef pituitary was added to 10 ml of spring water. The mixture was shaken, placed in a refrigerator for 24 hours, then centrifuged. The supernatant fluid was used as the stock solution. Of this, various amounts were added to the dishes containing the experimental pieces. One-tenth of one ml of stock solution to 10 ml of spring water was found to be optimum. In this concentration, regeneration was most rapid. Higher concentrations than this were lethal. Twenty-two regenerating animals in media containing the optimum amount of pituitary extract showed complete regeneration in 156 to 180 hours. A decrease in total area of about 50% accompanied the regeneration of head pieces. Tail pieces showed a reduction of about 30% in area when regeneration was complete. One-third of the head pieces and one-half of the tail pieces were found to have abnormally large proboscides. The controls, 54 in number, required a minimum of 236 hours for complete regeneration. The reduction in area of the controls was less than 5% at the time of complete regeneration; and no abnormally large proboscides were observed among the control animals.

To delimit further the causative factors of the accelerated regeneration in the presence of whole pituitary extract, 2 series of experiments were carried out. In one of these the experimental pieces were placed in media containing anterior lobe extract only. In the other series, extract of the posterior lobe alone were used. These preparations contained no preservative,[†] and were employed in dilutions of 0.01 ml to 1 ml per 10 ml of spring water. The animals were transected in the same manner as in the preliminary experiments.

With anterior lobe extract, 166 regenerating pieces were used. Of these, 42 died before showing any regeneration. However, among the 164 control pieces, 42 died so that the deaths among the experimental animals cannot be attributed to the media. The average time for complete regeneration of the planaria in media containing an-

[†] Anterior and posterior extracts supplied by Sharpe & Dohme, Inc., Philadelphia.

terior lobe extract was 450 hours whereas the controls required an average of 504 hours. The average reduction of area for the experimental pieces was 8.5% ; for the control pieces, 5%.

In the series of experiments with posterior lobe extract 160 regenerating pieces were tested. The time for complete regeneration for this series averaged 336 hours, an acceleration of 33% over the controls. There was an average reduction in area of 41% as compared with 5% for controls.

Toward the completion of regeneration the control animals divided by fission in 61% of the cases. No fission was observed in the experimental animals in either of the series with posterior and anterior pituitary extracts.

Measurements of respiratory activity after regeneration of 52 hours, in control medium and in concentration of 0.2 ml posterior pituitary extract, per 10 ml spring water gave a Q_{O_2} for the controls of 4.54. The Q_{O_2} for the experimental animals was 5.94.

These experiments indicate that regeneration is accelerated in the presence of pituitary extracts, and especially extracts of the posterior lobe. The experimental medium contained 0.17 mg of pituitary material per ml for the tests with posterior pituitary. This together with the fact that the animals decreased in size eliminates feeding as a complicating factor.

Summary. With extract of desiccated whole beef pituitary gland there was a mean increase of 35% in the rate of regeneration of bisected planaria (*Euplanaria dorotocephala*) with a decrease in total area in head pieces of 50% and a decrease of total area in tail pieces of 30%, when completely regenerated. Posterior lobe extract accelerated regeneration by 33% with a reduction in area of 36% over the controls. The acceleration of regeneration with anterior extract alone was not as marked as with the whole extract or the posterior fraction. There was no reproduction by fission which occurred in 61% of the controls.

Evidence for the Local Effect of Mercurial Diuretics.*

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Previous attempts to demonstrate "tissue diuresis" by "mercurial diuretics" failed since the investigated changes of blood colloids or salts were unconvincing. This paper describes certain new observations which may throw light upon the problem.

In previous experiments the inhibition of procaine convulsions by calcium salts was demonstrated.¹ Organic calcium salts were found to be particularly efficient.² Since magnesium salts were found to prevent the anticonvulsive action of calcium salts it seemed that the well known increase of membrane impermeability produced by calcium salts was the chief factor involved in their action.

This property of calcium salts is probably related to their *diuretic* action. Consequently, we were led to investigate other diuretics, particularly the strong acting mercurial diuretics, such as hydroxy mercuri-methoxy-propyl carbamyl phenoxy acetate, commonly known as "salyrgan". Our expectation was fully justified since salyrgan exhibited an anticonvulsive effect far exceeding that of any calcium salt. The invariably convulsive and oftentimes fatal dose of 200 mg/kg of procaine, intramuscularly in guinea pigs, was rendered entirely harmless by as little as 20 mg/kg of salyrgan simultaneously administered. For comparison, the most effective of all the previously tested calcium salts, calcium benzoate, had to be given in a dose of 50 mg/kg in order to detoxify 200 mg/kg of procaine.

The detoxifying action of salyrgan might be explained as the result of a direct chemical combination with procaine. Various other chemically different convulsant drugs were therefore studied, such as strychnine, picrotoxin, coramin and metrazol. In every instance salyrgan in doses of 10 to 20 mg completely inhibited the action of

* This investigation has been made with the assistance of a grant from the Committee on Therapeutic Research, Council of Pharmacy and Chemistry, American Medical Association.

¹ Beutner, R., and Miley, G. P., *Proc. Soc. Exp. Biol. and Med.*, 1938, **38**, 279.

² This work was done in collaboration with H. Wastl and A. Jensen (*Ibid.*, 1939, **42**, 547). See also the extensive publication of H. Wastl (*Arch. int. Pharmacodyn.*, 1939, **43**, fasc. 2) giving quantitative data.

a highly convulsant dose of these drugs. It seems unlikely that salyrgan can combine with any or all of these chemically widely different convulsant drugs. Moreover, various calcium salts such as calcium levulinate, were likewise found to inhibit all of these convulsants. This can hardly be the effect of a direct chemical combination.

The assumption that both salyrgan and calcium salts act primarily on the tissues rendering them more impermeable and *thus* preventing convulsions is further supported by the following findings:

(1) According to our observations no perceptible inhibition of convulsions occurs *in frogs* when salyrgan and strychnine, or another convulsant, are injected simultaneously in any lymph sac. Obviously there is a direct access to the nerve cells in this case, membrane permeability being of less importance in the loose tissue of the frogs. If the convulsions were inhibited by direct chemical interaction the inhibition should occur in the frog just as in the mammal injected intramuscularly.

(2) According to recently published experiments by Spiegel and Spiegel-Adolf³ convulsions are accompanied by an increase of the electrical conductivity of the brain tissue, pointing to an increased cell permeability brought about by the convulsing agent. Since calcium salts decrease permeability it seems more than likely that this decrease is the *very cause of their anticonvulsive action*. The same seems to be true for salyrgan. Since the decrease of permeability is likely to be associated with a dehydration of the tissue, the diuretic action of salyrgan would be explained on the same ground; in other words salyrgan should dehydrate tissues. The water thus eliminated would be ready for excretion through the kidneys.

In contrast to this theory of "tissue-diuresis" by salyrgan, the well known experiments by Govaerts⁴ support the assumption of a renal irritation, favoring glomerular filtration and hindering of tubular reabsorption, as the cause of salyrgan diuresis.

Would it be possible to explain the anticonvulsive action of salyrgan through its renal diuretic effect which possibly leads to an accelerated elimination of the convulsive drug through the kidney? If this were correct salyrgan should also prevent convulsions if injected separately prior to procaine, strychnine, etc. Experiments showed that such an effect does not occur, or, at any rate the inhibition of convulsions is very slight on separate injection.

³ Spiegel, E., and Spiegel-Adolf, M., *Proc. Soc. Exp. Biol. and Med.*, 1939, **42**, 834.

⁴ Govaerts, P., *Compt. rend. Soc. de biol.* 1928, **99**, 647.

The xanthin diuretics were also tested for their possible anti-convulsive action, but were found not to show any such action. Their diuretic effect would, therefore, seem to be of renal origin exclusively. Other heavy metal compounds, however, are likewise anticonvulsive, hence "tissue-diuretic". Detailed data are to be given later.

Conclusion. Mercurial diuretics have a potent anticonvulsive effect. Study of the details of this effect leads to the conclusion that these diuretics increase membrane impermeability, dehydrate tissue and thus work as "tissue diuretics," notwithstanding their well known renal action.

11376 P

Serum Albumin Changes in Hypoproteinemic Dogs Following Administration of Methionine or Phenylalanine.

K. S. KEMMERER AND G. P. HEIL. (Introduced by Warren M. Cox, Jr.)

From the Mead Johnson Laboratories, Evansville, Indiana.

The observation by Whipple and his coworkers that single amino acids and pure chemical substances increase the production of hemoglobin¹ and serum protein² raises the question of whether observed increases in serum protein after protein feeding are not solely the result of chemical stimulation, or mobilization from body stores of protein, as distinguished from a new synthesis from the ingested protein materials. If so, recorded differences in proteins (when concluded from changes in serum protein levels) would be merely an expression of a difference in amino acid composition and not necessarily express the nutritive value of the proteins for growth or maintenance.

Before such an interpretation can be made, it is essential to have additional data; and especially to determine whether single amino acids under conditions of low dietary protein, as in the procedure of Weech and Goettsch³ will effect an increase in serum protein.

¹ Robscheit-Robbins, F. S., and Whipple, G. H., *Proc. Soc. Exp. Biol. and Med.*, 1939, **41**, 361.

² Madden, S. C., Noehren, W. A., Waraich, G. S., and Whipple, G. H., *J. Exp. Med.*, 1939, **69**, 721.

³ Weech, A. A., and Goettsch, E., *Bull. Johns Hopkins Hosp.*, 1938, **63**, 154.

TABLE I.
Effect of Supplements of Methionine and Phenylalanine on Hypoalbuminemia
Induced by a Low Protein Diet.

Dog No.	Plasma albumin in g %				Supplement 1 g daily during reg.	Change in plasma albumin after 1-wk reg.
	Initial	End 3-week depletion	End 1-week regeneration	End 2-week reg.		
31	3.52	2.92	2.50	—	Phenylalanine	—,42
32	3.37	2.51	2.13	—	"	—,38
21	3.59	2.76	2.51	2.46	"	—,25
39	3.41	2.72	2.54	2.48	"	—,18
28*	2.76	2.03	1.96	—	"	—,07
36	3.08	2.48	2.45	2.28	"	—,03
29*	2.79	1.28	1.42	—	"	+ ,14
Potency value -0.02 ± 0.051						
29	2.63	2.16	2.19	2.19	Methionine	+ ,03
35	3.52	2.66	2.74	2.53	"	+ ,08
2	3.50	2.42	2.51	2.53	"	+ ,09
28	3.35	2.61	2.72	2.99	"	+ ,11
9	3.44	2.27	2.45	2.39	"	+ ,18
2*	3.40	2.27	2.55	—	"	+ ,28
9*	2.63	1.79	2.12	—	"	+ ,33
Potency value 0.307 ± 0.028						

*Indicates observations made Sept.-Oct., 1939; all others, Dec.-Jan., '39-'40.

Fourteen dogs were used for the study and the above procedure³ followed in all details. Total protein, albumin and non-protein nitrogen were run on the plasma from blood taken at the beginning and end of 3-week depletion on the low protein diet, and after one week regeneration. A few observations were made after 2 weeks' regeneration. Supplement consisted of 1 g dl-methionine* or 1 g dl-phenylalanine* added daily to the 53 g per kilo of the basal diet. Table I gives the summarized findings.

The 7 dogs fed 1 g methionine showed an average increase in serum albumin of 0.157 g %. If the Weech and Goettsch figure of 0.15 (to compensate for the fall in albumin that would have been observed during the 4th week if the supplement had not been fed) is added, the potency value of methionine becomes 0.307 ± 0.028 . Phenylalanine resulted in an average drop of -0.17 g %; to which is added 0.15 for a potency value of -0.02 ± 0.051 . Studies in this laboratory would assign a potency value of 0.476⁴ to casein. Thus, 1 g methionine under the conditions of the experiment resulted in almost as much increase in serum albumin as did casein, while phenylalanine was not at all effective.

* Purchased from Eastman Kodak Company; amino nitrogen (van Slyke) content of methionine 9.37% or 99.8% of theoretical; of phenylalanine 8.52% or 100.4% of theoretical.

⁴ Mueller, A. J., Kemmerer, K. S., Cox, W. M., Jr., and Barnes, S. T., *J. Biol. Chem.*, in press.

We have had no previous experience with 2 weeks' regeneration, and it is not a part of the Weech and Goettsch procedure. In this instance the change in serum albumin after 2 weeks' regeneration confirms the distinction between methionine and phenylalanine. Other amino acids are being studied in this manner and will be reported later.

11377 P

Role of Particulate Matter in Perfusion of Blood Vessels.*

BENJAMIN W. ZWEIFACH. (Introduced by Robert Chambers.)

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A series of perfusion studies were made in which the capillary vessels of the frog's mesentery were kept under observation through the microscope. Striking differences were found between the circulation obtained with perfusates containing particulate matter and that obtained with similar solutions which were particle-free. Colloidal Ringer perfusates free of particulate matter did not fill all the vessels of the capillary bed, circulating only through the a-v capillaries. In a previous publication,¹ it was pointed out that the a-v capillaries represent direct continuations of the arterioles and serve as bridging channels to the venules. The dye T-1824 (Evans blue, Eastman Kodak Co.) has been used for blood volume studies because of its poor diffusibility.² When solutions containing Evans blue were used, the restriction of the color to the a-v capillaries stood out in contrast to the true capillaries which remained colorless. The addition of particulate matter, either as a fine suspension of carbon or of washed, rooster red cells, to the Ringer-gelatin or Ringer-acacia perfusates altered the restricted circulation within 30 to 45 seconds by distributing the solution throughout all of the capillaries.

During the early stages of the perfusion with particle-free Ringer-gelatin solutions, it was observed that the true capillaries were quickly emptied of their contained blood cells. This was peculiar since other observations had shown that the circulation of such perfusates

* This study was made possible by a grant from the Josiah Macy, Jr., Foundation.

¹ Zweifach, B. W., *Anat. Rec.*, 1939, **74**, 475.

² Gregerson, M. I., and Gibson, J. G., *Am. J. Physiol.*, 1937, **120**, 494.

was sharply restricted to the a-v vessels. By observing the vessels at the commencement of the perfusion, the blood cells in the true capillaries were seen to move simultaneously towards both the arterial and venous ends of the vessels and to be swept into the a-v circulation. This phenomenon appeared to be brought about by a suction effect arising from the rapid streaming of the perfusate past the true capillary orifices in the walls of the a-v channels. The circulation of the particle-free perfusate remained limited to the a-v capillaries throughout the experiment. No change in the character of the circulation was obtained by raising the perfusion pressure from the normal level of 30 mm up to 75 mm Hg. The augmented pressure merely effected a more rapid streaming through the a-v capillaries.

The result obtained when the animal was perfused with Ringer-gelatin solutions to which a suspension of carbon or avian red cells had been added, was in marked contrast to the above. Under these conditions the circulating fluid not only coursed through the a-v capillaries, but streamed into all the capillary side branches. The repeated, alternate use of particle-free and particle-containing solutions in the same preparations emphasized the distinct difference in capillary circulation obtained with the two types of perfusates. The red cells, because of their larger size and extreme plasticity, were more effective in this respect than carbon. The particulate matter appeared to create a series of disturbances at the points of capillary branching, thereby disturbing the axial a-v current and deflecting the perfusate into the true capillary offshoots.

Ringer-gelatin mixtures, lacking formed elements in suspension, were capable of preventing edema for only 30 to 40 minutes. Ringer-gelatin solutions containing carbon, however, delayed the onset of edema for about 110 minutes. Red cell suspensions were somewhat more efficient, no edema occurring for more than 180 minutes.

An additional interesting feature of the perfusion with red cell suspensions was the part played by these cells in plugging leaks in the capillary wall. When the capillaries were perfused with artificial solutions for more than 120 minutes, the capillary wall tended to become increasingly porous. Chambers and Zweifach³ have also shown that temporary porous spots appear in the capillary wall and can be increased or decreased by variations in the pH and calcium content of the perfusate. In this stage, a characteristic flattening of red cells against the leaky portions of the wall was observed. This was often followed by portions of the cells being squeezed into tiny openings between the endothelial cells at these points.

³ Chambers, R., and Zweifach, B. W., *J. Comp. and Cell. Physiol.*, 1940, in press.

It is suggested that, as a result of the markedly restricted circulation with particle-free perfusates, abnormal conditions develop which alter the capillary wall and bring about excessive capillary permeability. This would account for the early appearance of edema with such solutions. The widespread distribution of particle-containing perfusates approached a more normal circulation in the capillary bed and was thereby instrumental in pronouncedly delaying the onset of edema.

11378

Attempt to Produce Experimental Cardiospasm in Dogs.*

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In the clinical condition of cardiospasm food does not pass readily from the esophagus into the stomach although at autopsy the cardia does not exhibit hypertrophy or stenosis.¹ Postmortem studies have shown degeneration of the vagi^{2, 3} and loss of ganglion cells⁴⁻⁹ from the myenteric plexus of the cardia. Failure of the normal receptive relaxation of the cardia in response to the swallowing of food is cited by Hurst¹⁰ as the cause of cardiospasm. Cannon¹¹ demonstrated that this mechanism is abolished in cats following section of the vagi in the neck. By cutting the vagi in the thorax Knight¹² was able to reproduce the X-ray appearance of cardiospasm in anesthetized cats. In the course of a study of the motility of the

* Aided by a grant from the John and Mary R. Markle Foundation.

¹ Sturtevant, M., *Arch. Int. Med.*, 1933, **51**, 714.

² Heyrovsky, H., *Arch. f. klin. Chir.*, 1913, **100**, 703 (Quoted by Lendrum, *loc. cit.*).

³ Loeper, M., and Forestier, J., *Aech. d. mal de l'app. digestif.*, 1921, **11**, 306. (See Lendrum, *loc. cit.*)

⁴ Hurst, A. F., and Rake, G., *Quart. J. Med.*, 1930, **23**, 491.

⁵ Cameron, J., *Arch. Dis. Childhood*, 1927, **2**, 358.

⁶ Beattie, W. J. H. M., *St. Bartholomew's Hosp. Rep.*, 1931, **64**, 39.

⁷ Mosher and McGregor, *Ann. Otol. Rhin. and Laryng.*, 1928, **37**, 12.

⁸ Lendrum, F. C., *Arch. Int. Med.*, 1937, **59**, 474.

⁹ Hara, H. J., *California and Western Medicine*, 1929, **30**, 390.

¹⁰ Hurst, A. F., *J. A. M. A.*, 1934, **102**, 582.

¹¹ Cannon, W. B., *Am. J. Physiol.*, 1904, **19**, 436.

¹² Knight, G. C., *Brit. J. Surg.*, 1934, **22**, 155.

cardia in dogs Zeller and Burget¹³ performed thoracic vagotomy in nine animals; subsequent studies without anesthesia revealed no loss of tonus or failure of relaxation. They suggest that vagal impulses may be transmitted to the cardia through fibers which travel in the wall of the esophagus.

The present report deals with an attempt to interrupt any such fibers by combining an encircling incision through the outer coats of the esophagus with bilateral thoracic vagotomy.

Since anesthetics interfere with visceral reflexes, a modification of the method outlined by Burget and Zeller¹⁴ for recording motility in nonanesthetized dogs was used. Employing this method, the dogs were subjected to preliminary esophagostomy in which the esophagus was brought to the exterior in the midline below the cricoid cartilage. These animals did not regurgitate nor did they lose any considerable amount of food during the act of swallowing. Following recovery the dogs were trained to lie quietly on a table while motility studies were made. Three rubber balloons attached to soft rubber catheters were passed down the esophagus, the lowest being lodged in the cardia, the second in the lower esophagus, and the uppermost a short distance below the esophageal fistula. The 2 lower balloons were connected to sensitive Becker tambours. The uppermost balloon was connected by a T-tube with a rubber bulb and with a mercury manometer. By means of this arrangement stimulation of the esophagus could be effected and recorded. Momentary distension of the uppermost balloon resulted in an almost immediate relaxation of the cardia, which definitely preceded the passage of a peristaltic wave over the middle balloon.

When the dogs were well trained the interruption of nerve pathways in the thorax was attempted. Under nembutal anesthesia and artificial respiration by tracheal catheter, the thorax was entered on the left side, both vagi and the communicating branch were cut about 4 cm above the diaphragm, and the outer coats of the esophagus were cut along its entire circumference at the same level, laying bare a band of white submucosa 1 cm wide. In 5 dogs this procedure was varied by doing the operation in 2 stages, first girdling the esophagus, then reëntering the thorax a few weeks later to cut the vagi.

After operation the animals were observed for difficulty in swallowing, presence or absence of cardiac relaxation, and change of

¹³ Zeller, W. E., and Burget, G. E., *Am. J. of Digest. Dis. and Nutrition*, 1937, **4**, 113.

¹⁴ Burget, G. E., and Zeller, W. E., *PROC. SOC. EXP. BIOL. AND MED.*, 1936, **34**, 433.

body weight. The dogs subjected to simple girdling of the esophagus alone did not experience difficulty in swallowing, and receptive relaxation of the cardia was present.

All the dogs subjected to the combined operation of girdling of the esophagus and thoracic vagotomy showed marked loss of weight and great difficulty in swallowing. A dog when given a half can of food would gulp it down, immediately begin to strain and extend its neck, and make swallowing movements and soon return practically all of the food as a mucous-coated, sausage shaped mass. The regurgitated food did not change the color of blue litmus paper. By repeated attempts the dog might dispose of half a can of food within 10 minutes. In this group of dogs 5 showed complete absence of relaxation of the cardia in all motility records taken postoperatively. Two dogs were too restless following the operations to permit taking satisfactory records, 2 revealed occasional slight relaxation in response to strong stimulation, and one exhibited relaxation which was indistinguishable from that demonstrated in preoperative records. In spite of recorded relaxation these last 3 animals exhibited marked difficulty in swallowing and rapid loss of weight. Of the 12 dogs used 2 died of perforation of the esophagus within 3 days following the thoracic operation. The results are summarized in Table I.

TABLE I.

Operation	No. of dogs studied	No. of dogs showing difficulty of swallowing	No. of dogs in which relaxation could be recorded
Encirclement of esophagus	5	0	5
Combined operation	10*	10	3†

*In 2 of these balloon records could not be obtained.

†Two of the 3 exhibited only occasional slight relaxation in response to strong stimulation.

On postmortem examination the 2 dogs with perforation of the esophagus showed mediastinitis. One died 13 days after operation with hemorrhages of undetermined cause into the stomach and small intestine. In this and other dogs sacrificed at intervals of 34 to 84 days after operation, the esophagus was found not to be inflamed, dilated or constricted. In every case one or 2 fingers could be passed easily through the cardiac orifice into the stomach.

The failure to demonstrate uniformly a complete loss of relaxation in 3 of the dogs is not readily explained. It may be that all vagal pathways concerned in the relaxation of the cardia were not severed. It is possible that the motility studies are capable of showing a relaxation of the cardia which is not sufficient in degree to allow the free passage of food into the stomach.

Summary. A combined operation of bilateral vagotomy 4 cm above the diaphragm and girdling of the esophagus at the same level is described as a means of producing in dogs an experimental condition comparable to clinical cardiospasm. In the majority of cases the regurgitation of food can be shown to be accompanied by a failure of receptive relaxation of the cardiac orifice of the stomach. Since neither bilateral vagotomy in the thorax¹⁴ nor girdling the esophagus above the diaphragm is sufficient in itself to produce these results, it may be concluded that some but not all of the fibers responsible for receptive relaxation of the cardia of the dog course downward within the wall of the esophagus.

11379

Failure of Maternal Vitamin A Depletion to Produce Congenital Anomalies in the Young of Rats.

MOTT D. CANNON. (Introduced by W. B. Cannon.)

From the Department of Child Hygiene of the Harvard School of Public Health.

Attention recently has been directed to possible dietary causes of congenital anomalies by Hale's observations^{1, 2, 3} on the occurrence of microphthalmia, hare lip, cleft palate, and the failure of the kidneys to leave their embryonic position in the young of vitamin A-deficient sows. Others who have investigated reproduction in vitamin A-deficient hogs have reported abortion, resorption or the birth of dead fetuses. In some instances prolonged labor was observed, but congenital anomalies in the young were not described.^{4, 5}

Hart, Meade and Guilbert⁶ made no mention of congenital defects in calves born to cows showing night blindness at the time of parturition, nor were anatomical abnormalities recorded by Hart and Miller⁷ among lambs from ewes kept on vitamin A-low rations for nearly a year and night blind at the time of lambing.

¹ Hale, Fred, *J. Heredity*, 1933, **24**, 105.

² Hale, Fred, *Am. J. Ophthal.*, 1935, **18**, 1087.

³ Hale, Fred, *Texas State J. Med.*, 1937, **33**, 228.

⁴ Hughes, J. S., Aubel, C. E., and Lienhardt, H. F., *Kansas Agric. Exp. Sta. Tech. Bull.*, 1928, **23**, 1.

⁵ Hughes, E. H., *J. Agric. Res.*, 1934, **49**, 943.

⁶ Hart, G. H., Mead, S. W., and Guilbert, H. R., *PROC. SOC. EXP. BIOL. AND MED.*, 1933, **30**, 1230.

⁷ Hart, G. H., and Miller, R. F., *J. Agric. Res.*, 1937, **55**, 47.

Sherman and MacLeod,⁸ Evans,⁹ and Batchelder¹⁰ all found reduced reproductive capacity in vitamin A-deficient rats. Sure¹¹ was the first to show that lack of vitamin A results in resorption of fetuses, even if abundant vitamin E is provided. This work was confirmed by Mason,¹² who showed that vitamin A deprivation also caused appearance of the "placental sign" (red blood cells in the vaginal smear) one to 4 days early, prolonged gestation, and difficult parturition with all or part of the young born dead. Mason makes no mention of congenital anomalies, although he states that all young born to his large series of vitamin A-deficient mothers were routinely examined. Tansley¹³ and Newton,¹⁴ who have confirmed Mason's work, observed no anomalies in the young.

Hale³ states that he has observed 2 cases of congenital blindness in rats from vitamin A-deficient mothers, but mentions that hereditary factors are not ruled out. Browman,¹⁵ investigating the reproductive performance of rats receiving adequate vitamin A but which had had a previous history of vitamin A depletion, encountered microphthalmia in one percent of the young born to these mothers. However, 0.7% of the young from the stock colony showed this defect.

In an extensive study of congenitally malformed children, Murphy and Bowes¹⁶ have judged the maternal diet to have been inadequate in 40% of the cases. They do not, however, present control studies on the incidence of inadequate diets among mothers of normal infants.

We have studied the effects of maternal vitamin A deprivation upon the rat fetus, placing particular emphasis upon the question of congenital anomalies.

Two A-low diets were employed:

	A	B
Casein (hot alcohol extracted)	24	22
Sucrose	20	64
Hydrogenated cottonseed oil (Crisco)	22	—
Salt mixture No. 185 ¹⁷	4	4
Yeast (ether extracted)	10	10

⁸ Sherman, H. C., and MacLeod, F. L., *J. Am. Chem. Soc.*, 1925, **47**, 1658.

⁹ Evans, H. M., *J. Biol. Chem.*, 1928, **77**, 651.

¹⁰ Batchelder, E. L., *Am. J. Physiol.*, 1934, **109**, 430.

¹¹ Sure, B., *J. Agric. Res.*, 1928, **37**, 87.

¹² Mason, K. E., *Am. J. Anat.*, 1935, **57**, 303.

¹³ Tansley, K., *Biochem. J.*, 1936, **30**, 839.

¹⁴ Newton, W. H., *J. Physiol.*, 1938, **92**, 32.

¹⁵ Browman, L. G., *Am. J. Physiol.*, 1939, **125**, 335.

¹⁶ Murphy, D. P., and Bowes, A. D., *Am. J. Obstet. and Gynec.*, 1939, **37**, 460.

¹⁷ McCollum, E. V., Simmonds, N., and Pitz, W., *J. Biol. Chem.*, 1916, **27**, 33.

Diet B was supplemented by 0.2 cc of cottonseed oil 3 times weekly.

Since performance on these 2 diets does not appear to differ in any important respect, the results will be discussed without reference to the diet used.

Females previously fed Purina dog chow were placed on the described diets at approximately 30 and 60 days of age. They were placed with males at varying periods after the vaginal smears had shown cornified cells continuously.

Of 36 females, mated as proven by the demonstration of sperm in the vagina, 2 failed to conceive, 11 resorbed their fetuses completely,* and 23 carried one or more young to term. These results are tabulated in more detail in Table I.

In our experience, females so depleted as to show xerophthalmia or weight loss generally will not mate. Of the animals mated, the most severely depleted resorbed their fetuses. Those rats which gave birth to young showed gestation periods from 23 to 25 days, with normal labor in some instances, and in others, with labor extending over 12 hours. This confirms the observation of Mason, Tansley, and Newton. It is noteworthy that a similar prolongation of pregnancy with failure of the birth mechanism is induced by the injection of anterior pituitary preparations into pregnant rats.¹⁸ In this latter instance, prolongation of gestation and failure of the

TABLE I.

Age when placed on A-low diet, days	Days on A-low diet	Matings	♀ failing to conceive	♀ which resorbed completely	♀ which carried 1 or more young to term	Young born or carried to term	% of young alive
60-70	60-70	7	0	1	6	52	76
	100-120	12	0	1	11	81	51
25-30	60-70	7*	2	4	1	3	100
	85-90 } †	7	0	2	5	38	52
	100 }	3	0	3	0	—	—
Controls: A-low diet and 40 γ carotene 3x weekly bred when 90-110 days old		3	0	0	3	23	100
Controls: Purina dog chow and lettuce		11	0	0	11	68	100

*Received 0.25 cc cod liver oil daily after 9-11 day of pregnancy.

†Refused to mate and lost weight. Given 120 γ carotene between 60 and 75 days on A-low diet.

* That they had conceived and implanted fetuses was proved by the occurrence of the placental sign.

¹⁸ Teel, H. M., *Am. J. Physiol.*, 1926, **79**, 170.

birth mechanism are associated with persistence of abnormal lutein tissue in the ovaries and failure of a new crop of follicles to ripen.

Young born normally or obtained by Caesarian section were minutely examined for abnormalities. The following specific points were checked: ears, eyes, nostrils, lips, roof of mouth, limbs and feet, tail, vertebral column, anus and genital papilla. Except when it was desired to rear the young, the presence of eyeballs was unequivocally established by dissection, and in most instances the viscera, especially the kidneys, were examined. All of the young from A-low mothers appeared anatomically normal except for one which had only the stump of a tail. About half the young were born dead. Resorption sites and embryos in the process of resorption were found even in mothers who bore mature young. The macerated fetuses represented various stages of development, but no anomalies were found where sufficient structure remained to permit of satisfactory examination.

The young reared by A-low mothers (only the less deficient animals would nurse young) were essentially normal in appearance, although somewhat underweight. No evidence of impaired vision was noted. All the young ceased to grow and developed severe xerophthalmia by the time they were 5 weeks old.

Control mothers on dog chow and on the A-low diet plus 40 μ g of carotene 3 times weekly gave birth to living young in every instance after gestation periods of 21 to 23 days. Two of the young from control mothers on dog chow were hydrocephalic.

From these observations, as well as from the work of others already mentioned, it may be seen that, if rat females have sufficient vitamin A to enable them to bear any young, their offspring are anatomically normal. Sheep and cattle appear to behave similarly. Hale emphasized the severity of depletion of his sows, and the possibility should not be overlooked that swine may be able to mate, conceive and carry fetuses when more severely depleted than can rats, sheep, or cattle. If this is true, it is possible that this larger store of vitamin A in rats, necessary for conception, permits the early critical stages of the embryo's development to proceed unaffected.

Summary. 1. Lack of vitamin A failed to induce congenital anomalies in the young of rats. 2. Very severely depleted female rats refuse to mate. In order of decreasing severity of depletion, they will: mate but fail to conceive; conceive but resorb their fetuses; give birth to dead fetuses with difficult and protracted labor after a prolonged gestation; show only prolonged gestation.

**N'Dodecanoylsulfanilamide, and Sulfapyridine Plus Vitamin C,
in Experimental Tuberculosis in Guinea Pigs.***

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The striking chemotherapeutic effects obtained recently by the use of sulfanilamide and related compounds have led many workers to study the therapeutic effects of these chemicals on experimental and clinical tuberculosis. The findings reported have been conflicting, and the search for a chemotherapeutic agent which would act efficiently in tuberculosis is still being continued.

As early as 1920, the fatty acids of chaulmoogra oil were suggested for the treatment of tuberculosis (Walker and Sweeney¹), and clinical success in the treatment of skin tuberculosis with hydnocarpic acid has been reported by Rogers² and by Burgess.³ Kolmer and his associates,⁴ however, showed that chaulmoogric acid does not inhibit the course of experimental tuberculosis in guinea pigs. Recently Crossley, Northey, and Hultquist⁵ synthesized N'dodecanoylsulfanilamide with the thought that a combination of a long chain fatty acid with sulfanilamide might provide a more effective chemotherapeutic agent for tuberculosis than either constituent alone. When this compound was administered to guinea pigs by stomach tube in 100 mg amounts daily for 45 days after infection with H37, Climenko and Schmidt⁶ reported no sign of generalized tuberculosis, as evidenced by the absence of gross involvement of liver or spleen or of general lymphadenitis in these animals, 120 days following infection. Through the courtesy of Dr. D. A. Bryce⁷ we were supplied with generous amounts of N'dodecanoylsulfanilamide for the purpose of studying its action in experi-

* This work was in part supported by a grant from the National Tuberculosis Association.

1 Walker, E. L., and Sweeney, M. A., *J. Inf. Dis.*, 1920, **26**, 238.

2 Rogers, L., *Brit. Med. J.*, 1933, **1**, 47.

3 Burgess, W., *Brit. Med. J.*, 1935, **2**, 835.

4 Kolmer, J. A., Davis, L. C., and Jager, R., *J. Inf. Dis.*, 1921, **28**, 265.

5 Crossley, M. L., Northey, E. H., and Hultquist, M. E., *J. Am. Chem. Soc.*, 1939, **61**, 2950.

6 Climenko, D. R., and Schmidt, R. L., personal communication.

7 Bryce, D. A., Medical Director, Calco Chemical Co.

mental tuberculosis. Throughout the course of this work, tuberculin-negative, male guinea pigs were used.

Experiment I. Eighteen guinea pigs were infected subcutaneously in the region of the groin with 1 mg of human type tubercle bacilli (H37) and divided into 3 groups of 6 animals each as follows:

Group I. Untreated controls.

Group II. Treated with 100 mg N'dodecanoylsulfanilamide in 2% olive oil solution, administered by stomach tube. Treatment started on the day of infection and continued daily for 45 days.

Group III. Treated with 100 mg N'dodecanoylsulfanilamide in 2% olive oil solution, administered by stomach tube. Treatment started 5 days after infection and continued daily for 40 days.

Results. Three control animals died 40 days after infection, 1 animal of Group II died 25 days after infection, and 2 animals of Group III died 40 days after infection. The remaining guinea pigs were sacrificed 60 days after infection. The macroscopic findings, averaged for severity of involvement, are presented in Table I.

TABLE I.
Extent of Tuberculosis.

Group	Lungs	Liver	Spleen	Size of spleen	Glands	Summary
I	1.6+	3.0+	2.5+	4.6 × normal	2.1+	2.3+
II	1.1+	2.1+	2.1+	3.5 × "	2.1+	2.0+
III	1.2+	2.4+	2.6+	4.8 × "	2.6+	2.2+

Although the treated animals showed somewhat less disease than the control pigs, there was no evidence of localization and the differences were very slight indeed.

The experiment was, therefore, repeated with a smaller infecting dose. For purpose of comparison, groups of animals treated with sulfapyridine and with sulfapyridine plus vitamin C were included in this series. The latter were added in view of the favorable results obtained previously with vitamin C treatment of experimental tuberculosis.⁸

Experiment II. Thirty-five guinea pigs were infected subcutaneously in the region of the groin with 0.1 mg of H37 and divided into 5 groups of 7 animals each, as follows:

Group I. Untreated controls.

Group II. As in Experiment I.

Group III. As in Experiment I.

Group IV. Treated with 100 mg sulfapyridine in 2% olive oil solution, administered by stomach tube. Treatment started on the day of infection, and continued daily for 45 days.

⁸ Steinbach, M. M., and Klein, S. J., in preparation.

Group V. Treated with 100 mg sulfapyridine in 2% olive oil solution, administered by stomach tube, and 10 mg vitamin C dissolved in normal saline, administered subcutaneously. Treatment started on the day of infection and continued daily for 45 days.

Results. One control animal died 33 days, and 1 died 120 days after infection. One animal in Group III was killed accidentally the day after infection. Two animals in Group IV died 140 days after infection, and 1 guinea pig in Group V died 120 days after infection. Three of the animals in each group were sacrificed 62 days after infection. The findings at this time were the same as obtained in Experiment I. The rest of the animals were sacrificed 140 days after infection. The average autopsy scores, including all the animals of each group, are presented in Table II.

TABLE II.
Extent of Tuberculosis.

Group	Lungs	Liver	Spleen	Size of spleen	Glands	Summary
I	2.3+	3.0+	3.9+	6.3 × normal	3.5+	3.2+
II	2.3+	3.0+	2.2+	3.0 × "	2.9+	2.6+
III	3.0+	3.4+	3.4+	5.0 × "	2.6+	3.1+
IV	2.5+	3.5+	2.5+	4.7 × "	2.6+	2.7+
V	2.7+	3.4+	3.4+	5.2 × "	3.0+	3.1+

In all groups the infection had spread to every susceptible organ of the guinea pig. The differences which exist in gross appearance and size of spleen between treated and untreated animals cannot be considered significant in view of Corper's⁹ report that this effect may be due to the toxic action of the drug. The general impression gathered at the autopsy table was that no group of animals showed significant differences in the extent or degree of tuberculous infection.

Conclusions. (1) Under the conditions of our experiments treatment with N'dodecanoylsulfanilamide shows no inhibitory effect on the course of experimental tuberculosis in guinea pigs infected with the human tubercle bacillus. (2) The administration of sulfapyridine, alone or in conjunction with vitamin C, is ineffective in the treatment of guinea pigs infected with the human tubercle bacillus.

⁹ Corper, H. J., Cohn, M. I., and Bower, C., *Am. Rev. Tuberc.*, 1939, **40**, 452.

On the Rôle of Oxalic Acid in Blood Clotting.

R. H. K. FOSTER. (Introduced by E. Chargaff.)

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Oxalic acid has always been considered an anticoagulant but recently Steinberg and Brown¹ and Schumann² reported that *small* doses administered intravenously hasten coagulation. Oxalic acid is a constituent of normal human blood ranging from 2.75 to 4 mg %³⁻⁶ and somewhat higher in rabbit and beef blood. Steinberg and Brown¹ and Steinberg⁷ stated that oxalic acid and plant extracts containing oxalic acid were found effective in increasing the coagulability of normal blood and also of blood in hemophilia, purpura, obstructive jaundice, vitamin K deficiency, and in prolonged post-surgical bleeding from other causes. (Reference 7 is a popular article by Hannah Lees describing Steinberg's work and so far is the most extensive source of information.) No data were presented by Steinberg concerning the action in vitamin K-deficient animals. Having available vitamin K-free chicks we decided to make a few tests. The effect in normal rabbits was also studied.

Steinberg stated that a coagulant "unit" was the amount of plant extract causing in a 5 lb rabbit 15 minutes after injection a reduction in the clotting time of 50%. He did not give the dosage of oxalic acid for rabbits, but mentioned the human dosage to be 3 mg.⁷ A corresponding dosage in proportion to body weight, with a considerable range on either side, was used in rabbits and chicks.

Coagulation times were determined by a modified Howell method. Blood was drawn into an oiled syringe and transferred to a vaccine tube which was immediately stoppered with a paraffined cork and placed in a water bath. The tube was tilted every half minute until clotting occurred. From chicks 0.2 cc of blood was drawn from the brachial wing vein and from rabbits 1.0 cc by heart puncture.

It is well known that after repeated veni- or heart-punctures increased coagulability may sometimes be observed. Controls were run to rule out this factor and the data are listed in Tables I and II.

¹ Steinberg, A., and Brown, W. R., *Am. J. Physiol.*, 1939, **126**, 638.

² Schumann, E. A., *Am. J. Obst. and Gyn.*, 1939, **38**, 1002.

³ Magerl, J. F., and Rittmann, R., *Klin. Wochschr.*, 1938, **17**, 1078.

⁴ Merz, K. W., and Maugeri, S., *Z. Physiol. Chem.*, 1931, **201**, 31.

⁵ Suzuki, S., *Jap. J. Med. Sci., II Biochem.*, 1934, **2**, 291.

⁶ Kamiya, S., *Jap. J. Med. Sci. II Biochem.*, 1937, **3**, 163.

⁷ Lees, Hannah, *Collier's*, 1939, Sept. 23, p. 48.

TABLE I.
Clotting Tests on Rabbits.

Animal No.	Dose per kg	Clotting times	
		Before	15 min after
303	Control—no injection	2+	3+
306	" " "	3	3+
317	" " "	3	2+
318	" " "	2+	3
319	" " "	3	2+
304	Saline— $\frac{1}{2}$ cc	3	3+
307	" $\frac{1}{4}$ "	2+	4+
325	Oxalic acid—10 γ	2+	2+
326	" " 20 "	3	2+
328	" " 40 "	3	4+
322	" " 60 "	2	2+
261	" " 80 "	4	2+
280	" " 90 "	3	3+
314	" " 125 "	2+	2+
270	" " 175 "	3	3
315	" " 250 "	3	2+
304	" " 450 "	3	3+
309	" " 600 "	3	> 5 hours

+ indicates half minute.

TABLE II.
Clotting Tests on Chicks.

Group No.	Condition of chicks	Dose γ /chick	N	Clotting times—min
1-3	Normal	Saline 0.02 cc	9	2+, 2+, 3+, 3+, 5+, 4+, 4+, 7, 7
2-4	"	Oxalic acid 2 γ	9	1+, 2, 2+, 2+, 4, 5+, 5+, 7, 7+
5-19-22	K-free	Control	12	88, 106, 10 >120
6	"	Venipuncture	4	All >120
7-20-23	"	Saline 0.05 cc	13	31 12 > 120
8	"	Oxalic acid 1 γ	3	All >30
9-10-11	"	" " 2 "	5	50, 92, 105, >30, >180
12	"	" " 3 "	3	All >30
24	"	" " 3.5 "	5	All >30
13-14-15-21	"	" " 5 "	11	18, 25, 99, 8 >120
16-17	"	" " 10 "	4	41, 46, 2 >120
18	"	" " 25 "	2	50, 73

A dose of oxalic acid of 40-50 γ /kg corresponds to a 3 mg human dose. As seen in Table I this dose was without effect in normal rabbits and the only significant effect obtained was with a dose of 600 γ /kg, which caused a great prolongation of the clotting time. Data on 8 other rabbits are omitted from the table to save space. The results were no different. Since the normal value of oxalic acid in rabbits is 6-9 mg %⁴ a 600 γ dose would correspond to an increase in the oxalic acid content of the blood of about 15%. The dose

recommended (40-50 γ /kg) as causing a shortening of the clotting time amounts to scarcely a 1% increase in the oxalic acid concentration. It hardly seems logical that so small an increase could affect the clotting time in either direction unless there was *no free oxalate* in the blood to start with. But whether the normal oxalate is combined or free, or whatever may be its function in blood, these data do not support the view that it possesses any *coagulant* effect whatsoever in normal blood.

Table II gives tests on normal and vitamin K-free chicks (Almquist⁸ diet). The chicks were 2 weeks old at the time of testing and most of them showed the characteristic hemorrhages caused by prothrombin deficiency resulting from an inadequate vitamin K supply. Doses of oxalic acid varying from 1 γ to 25 γ per chick were used and in no case was the clotting time reduced to normal. A dose of 3.5 γ per chick corresponds to the human dose given by Steinberg. Two chicks showed clotting times of less than 30 minutes, but this is not significant since controls occasionally showed similar results. Although one of the saline controls clotted at 31 minutes, the majority showed no clotting within 2 or 3 hours. Administration of vitamin K returned the clotting time to normal (not included in table). In normal chicks a dose of 2 γ per chick had no effect. The chicks used in the entire series ranged in weight from 50 to 145 g with an average of 98 g for 83 chicks.

Rabbit blood made deficient in coagulability by administering near threshold doses of heparin intravenously was unaffected by small intravenous doses of oxalic acid. With the doses of heparin used the clotting time returned to normal in about one hour. Heparin is generally considered as antiprothrombin and the heparinized animal is therefore comparable to the vitamin K-deficient chick, prothrombin being absent (or greatly diminished) in the latter and immobilized in the former. Only a few tests were run, and while the data were limited, there was no indication of any effect in the doses used nor evidence warranting more extended trials.

The literature describes oxalic acid as being a normal constituent of blood and other body tissues. The concentration changes in various conditions but there is no evident parallelism to changes in coagulation. During narcosis it increases in bile and in the urine.^{9, 10} This increase is attributed to anoxemia and Kamiya¹¹ and others

⁸ Almquist, H. J., *J. Biol. Chem.*, 1936, **114**, 241.

⁹ Borgstroem, S., *Skand. arch. Physiol.*, 1937, **77**, 16.

¹⁰ Borgstroem, S., *Skand. arch. Physiol.*, 1938, **79**, 1.

¹¹ Kamiya, S., *Jap. J. Med. Sci. II, Biochem.*, 1937, **3**, 301.

showed that anoxemia in rabbits increased the oxalic acid content of blood by as much as 60%. Kamiya⁶ and Marcolongo¹² showed that it rose in high blood pressure, uremia, tuberculosis, syphilis, beri beri, neuralgia, rheumatism, cirrhosis, and in acute and chronic nephritis. Melocchi¹³ observed a rise of oxalic acid in the blood during intestinal fermentation of carbohydrates. Olson also made the comment in a letter quoted by Schumann² that on the basis of the amount of oxalic acid normally in the blood, it would not seem logical that a small increase could have any effect on the clotting time. The experimental evidence bears out Olson's view.

Conclusions. Oxalic acid injected into animals over a wide range of dosage was found to have no effect on coagulation until a sufficiently high dose level was reached, at which point clotting was delayed. Included in this study were suitable controls, normal and heparinized rabbits and normal and vitamin K-deficient chicks.

11382 P

Direct Observations on the Circulation of Blood in Transilluminated Mammalian Spleens.*

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The spleens of living mice, rats, rabbits, guinea pigs and cats were transilluminated¹ and observed at several magnifications, as high as 600 \times (water immersion). Each type of spleen was completely delivered through a long paracostal incision and placed in a suitable celluloid chamber, on a light table, above the abdominal wall. By this means, respiratory motions were eliminated in some, and greatly reduced in all specimens. The spleen, thus supported, was totally immersed in rapidly flowing Ringer-Locke solution at 37°-38°C. Anesthesia hypodermoclyses of sodium iso-amyl ethyl barbiturate

¹² Marcolongo, F., *Clin. med. ital.*, 1934, **65**, 1068.

¹³ Melocchi, W., *Giorn. clin. med.*, 1934, **15**, 1669.

* The cooperation of the Department of Pathology, greatly facilitated this work.

¹ Knisely, M. H., *Anat. Rec.*, 1938, **71**, 503.

(sodium amytal, Lilly) provided adequate narcosis without disturbing the animal's position. Methods were devised for variously stimulating the spleen during the period of observation. The albino mouse was studied in greatest detail. It was noted, however, that the circulatory mechanisms of all species investigated presented fundamental similarities, which seemed to justify the following generalizations.

The afferent capillaries of the pulp communicate with naked pulp spaces at the point where their terminations are marked by ampullary dilatations and diminished mural refractility. No preformed, intactly lined connections between the arterial and venous systems in the spleen have been as yet conclusively demonstrated in our preparations. The break in vascular continuity is obvious in a relaxed or distended spleen, where the walls of the pulp channels are seen to be composed of spherical, oval or polyhedral cells and vague, linear shadows, suggestive of reticulum fibers; but, when the spleen is contracted, it is difficult to detect, because the residual channels, surrounded by compressed pulp cells, then assume the appearance of completely walled vessels, connecting arterial capillaries with venous sinuses and intralobular veins.

The capacity of the pulp is a function of capsular contraction, which takes place rhythmically as well as in response to specific stimulation. The behavior of the individual pulp spaces is additionally governed by arterial and venous blood-pressure variations, relationships to neighboring channels, the obstructing action of migrating leucocytes, derived either from the pulp cords or from associated arterial capillaries, and by swelling or shrinkage of the reticular stroma.

The pulp space is the most variable structure in the transilluminated spleen. It may convey, at different times, plasma almost devoid of red cells, rapidly flowing blood of normal cellular content, or slowly oozing, highly concentrated red cells. When the narrower, efferent end of the pulp channel is blocked by transient leucocytes, or by some other factor, blood accumulates within it. If, at this time, the adjacent pulp cells happen to be compressed, the channel becomes distended, and the blood within it acquires such concentration that the contours of individual erythrocytes are scarcely visible. In the absence of pulp compression and concomitant arterial constriction, the column of blood is obstructed momentarily, but rapidly alters its course and penetrates alternative passages to the nearest venous tributary. In our experience, this intermittency of circulation in the pulp most closely resembles, on a much smaller

scale, the cyclical blood flow observed by Knisely^{2, 3, 4} in the venous sinuses.

The venous sinuses and intralobular veins are passive recipients of blood. Their walls reveal innumerable stigmata, offering little or no obstruction to the influx of erythrocytes, and permitting the passage of lymphocytes and larger white cells with varying degree of distortion. In the absence of extrinsic interference with venous drainage, the sinuses swiftly transmit to the larger venous tributaries the blood which enters them from the pulp spaces. The only vascular sphincteric action that we have observed in the spleen pulp is exhibited by the arterioles and arterial capillaries, which are intermittently constricted, either individually or in groups.

Agonal vascular disintegration, as described by Knisely,⁴ has not been apparent in our preparations. Because of extreme capsular contraction, in the absence of positive arterial blood-pressure, the pulp of the dying spleen pales to a degree that is never otherwise attained. Having seen intracellular erythrocytes in living, normal splenic pulp, we are not satisfied with Knisely's assumption that phagocytosis of red cells is, in this situation, a purely agonal phenomenon. In effect, the spleen pulp acts as a filter, separating red cells from plasma, and removing foreign particulate matter, including certain erythrocytes, from the circulating blood. We have watched the appearance of India ink particles, inside pulp phagocytes, less than 20 seconds following their peripheral intravenous injection.

From the standpoint of reservoir function, the reaction which might be termed the 'emergency response' of the spleen, as classically demonstrated in the gross by Barcroft^{5, 6} and his colleagues, was consistently reproduced in our experiments by such factors as electrical stimulation of the pedicle nerves, adrenalin, exercise, anoxemia, hemorrhage and temperature changes. Microscopically, the response consisted of pulp compression and arterial constriction, leading to obliteration of the extravascular spaces and consequent mobilization of a quantity of relatively concentrated red cells.

Because of the pitfalls inherent in the technic of living tissue transillumination, our conclusions are necessarily tentative. We are reasonably convinced, however, that the circulatory systems of the spleens we have examined are 'open'; that, in other words, they lack

² Knisely, M. H., *Proc. Soc. Exp. Biol. and Med.*, 1934, **32**, 212.

³ Knisely, M. H., *Anat. Rec.*, 1936, **65**, 23.

⁴ Knisely, M. H., *Anat. Rec.*, 1936, **65**, 131.

⁵ Barcroft, J., *Lancet*, 1925, **1**, 319.

⁶ Barcroft, J., *Lancet*, 1926, **1**, 544.

the type of connection which commonly links arterial and venous capillary networks; that the pulp space—not the venous sinus—is the primary physiological unit of the splenic vascular mechanism; and that contraction of the capsule and trabeculae may convert the structurally 'open' circulation of a relaxed or distended spleen into a functionally 'closed' circuit.

11383

Electrical Method for Studying Water Metabolism and Translocation in Body Segments.

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Although the electrical resistance of a mummified arm would, obviously, be very much greater than that of a fresh cadaver, no serious attempt has ever been made to measure the water content of a body segment in terms of its electrical resistance. One of the reasons for this would appear to have been the lack, until comparatively recently, of technics which would permit resistance measurements to be made. Using alternating current, it has now become possible to measure, with a fair degree of accuracy, not only the resistance of various parts of the arm-to-arm segment in man (the upper arm, the chest segment alone or the arm and chest segments together) but also changes in their dielectric properties.^{1, 2, 3}

Hydration. In order to test out the possibility of measuring body water changes electrically, a liter of isotonic saline solution was injected intravenously into a normal individual weighing 50 kg who had been deprived of fluids for 3 hours previously and the electrical resistance of the arm-to-arm segment was measured before and 30 minutes after the injection. Resistance measurements were made by the immersion method at room temperature. The subject stood before a table 32 inches high and immersed the forearms in 9 liters of normal saline (11 cm deep) contained in a pair of arm baths supported on the table so that (1) the elbows rested on the arm-bath bottoms and (2) the upper arms were in a substantially

¹ Horton, J. W., and Van Ravenswaay, A. C., *J. Frank. Inst.*, 1935, **220**, 557.

² Barnett, A., and Bagno, S., *Proc. Soc. Exp. Biol. and Med.*, 1937, **36**, 543.

³ Barnett, A., *West. J. Surg.*, 1937, **45**, 380.

vertical position. Current was supplied to the arm baths at 11,160 cycles/second by the measuring instrument.³ About 90% of the resistance measured by this method is due to the unimmersed segment.⁴ Measurements over a control period of 2 hours were reproducible within $\pm 2\%$ and were made one minute after immersion.

The test subject excreted 150 cc of urine during the injection period (1 hour). Although the volume of retained saline (about 850 cc) was less than 2% of the total body weight (50 kg), a decrease of 10% in the measured resistance was observed. Confirming tests on a number of additional subjects yielded changes of the same order.

This surprisingly large decrease in body resistance demanded an explanation and the following working hypothesis was adopted. It is known that, when isotonic saline is injected intravenously, it does not distribute itself uniformly throughout the tissues but, after leaving the vascular system, accumulates in the extracellular or interstitial spaces.⁵ Since these spaces are estimated to constitute only 20-30% of the total tissue volume,⁵ a given volume of saline entering an extremity such as the arm, either fills in or increases the cross-section of the interstitial spaces by 3-5 times the amount that it would if it were also taken up intracellularly. The interstitial spaces in the arm lie in parallel relation to the muscle fibers. Current flowing along the arm splits up into parallel paths, part flowing along the inside of the muscle fibers and the rest along the interstitial spaces outside the latter. In the chest segment the current splits to flow in parallel paths through the chest muscles on the one hand and through the lung on the other. The reduction in resistance of the interstitial branches of these parallel circuits is the cause of the comparatively large resistance drop.

The distribution of extracellular isotonic saline following intravenous injection is variously reported in the literature. Skelton,⁶ working with cats, found that none of the saline reached the muscles but was absorbed mainly by the skin. Hastings and Eichelberger,⁷ on the contrary, working with dogs, traced considerable saline into the extracellular spaces of the muscles. The subject has been reviewed by Adolph.⁸ From the electrical standpoint, whether the saline accumulates between the muscle fibers or in the subcutaneous

⁴ Horton, J. W., Van Ravenswaay, A. C., Hertz, S., and Thorne, G., *Endocrin.*, 1936, **20**, 72.

⁵ Peters, J. P., *Body Water*, C. C. Thomas, Baltimore, 1935.

⁶ Skelton, H. P., *PROC. SOC. EXP. BIOL. AND MED.*, 1925-6, **23**, 499.

⁷ Hastings, A. B., and Eichelberger, L., *J. Biol. Chem.*, 1937, **117**, 73.

⁸ Adolph, E. F., *Physiol. Rev.*, 1933, **13**, 336.

tissues, the current path still remains a parallel one and the infiltrated saline shunts the intracellular branch of the circuit.

Since the cell walls in the arm are known to be dielectrics having capacitative properties, a change in arm resistance should modify the Q -factor which is the ratio between the reactances, due to these capacitances, and the resistance. Q -factor measurements were, therefore, made of a 10-cm length of the deep tissues of the right upper arm by the 4-electrode technic^{1, 2, 3} before and after the injection of a liter of saline.

Results are given in Table I for 10 subjects on whom measurements of both resistance and Q -factor were made. Nine of these subjects were mental patients with negative physical findings.

The resistance changes vary from 9 to 17% with a mean of 11.4% and the Q -factors from 12 to 27%—mean 17.5%. Normal subject A.B. is remarkable for the large changes in the electrical values despite the retention of only 440 cc of saline. Subject C.P. was given an injection of 2 liters of saline over a period of 2 hours. 1250 cc were retained. The resistance change was 60 ohms and the Q -factor decreased 0.028 (27%). Subjects C.P. and M.B. were given injections of one liter of Ringer's solution instead of isotonic saline. No significant difference in the order of the electrical changes was observed.

Resistance measurements were made on 3 subjects $3\frac{1}{2}$ hours and 24 hours after the end of the saline injection. The resistances after the $3\frac{1}{2}$ -hour interval were still decreased but had moved back towards the preinjection level roughly in proportion to the volume of urine excreted which took place at the rate of approximately 100 cc per hour. At the end of 24 hours, the resistances had returned to the preinjection levels.

Blood pressure measurements made before, after and during saline injections showed variations of the order of ± 10 mm which may be considered as not significant.

Dehydration. As a test of the reversibility of resistance measurements, a female subject weighing 63 kg and normally excreting 1500-1700 cc of urine daily, was put on a restricted fluid intake of 500 cc daily and 50 cc of 50% glucose was given intravenously twice daily for 3 days. Her body resistance was 200 ohms at the beginning of the experiment. On the fourth day, there had been a total weight loss of 2 kg, the urine output had descended to 600 cc for the previous 24-hour period and the body resistance had risen 45 ohms, from 200 to 245 ohms.

Clinical Applications. (1) A female patient 16 years of age and

TABLE I.
Effect of Intravenous Injection of One Liter of Isotonic Salt Solution on the A.C. Resistance of the Arm to arm Segment and on the Q-factor of a 10 cm Arm Segment.

Subj.	Sex	Age	Wt in kg	Saline retained cc	Resistance in ohms by the immersion method			Q-factor of a 10 cm arm segment		
					Before injection	After injection	% difference	Before injection	After injection	% difference
L.G.	♂	15	40.2	900	275	245	10.9	.066	.058	12.1
M.W.	♂	27	46.9	960	235	205	12.8	.106	.077	27.4
J.M.	♂	23	44.0	700	306	275	10.1	.070	.058	17.2
M.B.	♀	25	53.6	975	315	282	10.5	.078	.064	17.9
R.N.	♀	32	63.4	800	210	182	13.3	.078	.068	12.8
C.D.	♀	19	64.1	750	225	200	11.1	.101	.089	11.9
A.B.	♀	26	43.6	440	345	314	9.0	.081	.063	22.2
C.P.	♀	43	50.7	850	290	262	9.6	.102	.080	21.6
I.D.	♂	21	64.9	900	221	200	9.5	.123	.102	17.1
L.B.	♂	20	50.9	800	234	194	17.1	.102	.087	14.7
					Mean 11.4%			Mean 17.5%		

weighing 82 lb diagnosed as an hysteric had lost weight progressively as a result of prolonged vomiting. The vomiting episodes lasted for periods of several days during which time the patient regurgitated all food and liquid immediately after ingestion by inserting her fingers into her mouth to induce the vomiting reflex. The body resistance measured by the immersion method after 2 days of continual vomiting was the highest ever observed in a human subject—445 ohms. Suddenly the vomiting ceased and the patient ingested large quantities of food and liquid over a period of 48 hours. There was a gain in weight of $1\frac{1}{2}$ kg. At this point the body resistance was found to be 405 ohms. An injection of one liter of isotonic saline was then given over a period of one hour intravenously. 745 cc were retained. The resistance fell from 405 to 334 ohms, a change of 71 ohms.

(2) Resistance measurements were made in 2 cases of involutional melancholia (females) under treatment with testosterone propionate which is now widely used for menopausal disturbances.⁹ 25 mg were given intramuscularly thrice weekly. Zuckerman and Bourne¹⁰ have shown that, in primates, injections of testosterone propionate cause water retention. Recently Kenyon¹¹ and his co-workers have reported that this substance also produces water retention in man (normals). In both cases under treatment a progressive drop totaling about 10% in body resistance was observed in the course of a month. The absolute changes were 290 to 260 ohms and 285 to 251 ohms. Spontaneous resistance changes of this magnitude are not observed in conditions of this kind.

(3) Transverse resistance measurements were made on a female exhibiting a cyclic oedema of the ankles coincident with each menstrual period. The circumference at the ankle level increased by 4 cm during periods. Concentric electrodes^{12, 13} were mounted on the leg by means of rubber bands at a level 3 inches above the ankle so that the current passed diametrically from the internal to the external lateral sides and the resistance of the deep tissues between electrodes was measured at, and 2 weeks after, a period. The measured resistance increased from 118 during oedematous infiltration to 212 ohms, when the oedema had been resorbed.

Remarks. It is important to note that both the resistance and Q-

⁹ Kurzrock, L., Birnberg, C. H., and Livingston, S., *Endocrin.*, 1939, **24**, 347.

¹⁰ Zuckerman, S., Palmer, A., and Bourne, G., *Nature*, 1939, **143**, 521.

¹¹ Kenyon, A. T., Knowlton, K., Sandiford, I., Koeh, F. C., and Latwin, G., *Endocrin.*, 1940, **26**, 26.

¹² Barnett, A., *J. Physiol.*, 1938, **93**, 349.

¹³ Barnett, A., *West. J. Surg.*, 1937, **45**, 322.

factor decrease following infusion of isotonic saline. This is to be expected in a parallel type of circuit. In a circuit of the series type, a decrease in resistance would result in an increased Q . The studies here presented are of a preliminary nature and do not permit a judgment as to the contributory rôle of the various anatomical elements lying along the current path to the total result. A rough analysis of the probable relative effects of the extracellular and intracellular phases has been presented for the particular case where isotonic saline is injected intravenously. It is possible that vascular factors also intervene. Studies along these lines are now in progress.

I am indebted to A. N. Mayers for assistance during part of this investigation.

11384

Action and Toxicity of Vitamin B₆ Hydrochloride.

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In view of the increasing interest in vitamin B₆¹⁻⁴ as a nutritional accessory, the present investigation was undertaken in order to determine the degree of potency and limits of toxicity. All experiments were carried out with crystalline vitamin B₆ hydrochloride, m.p. 212°C (corrected) with effervescence.

General Properties. Vitamin B₆ · HCl is easily soluble in water. Its aqueous solutions are acid in reaction. A 1% solution has a pH of 2.44. *In vitro* a quantity of 4 mg caused hemolysis of sheep's washed erythrocytes, but if it was previously neutralized, no laking took place. Obviously the hemolytic effect was due to the acidity. When a 1% solution of B₆ · HCl in the amount of 0.5 cc was injected both subcutaneously and intramuscularly into 3 rabbits, practically no irritation occurred, but when 0.1 cc of the same

¹ György, P., *Nature*, 1934, **133**, 498; *J. Am. Chem. Soc.*, 1938, **60**, 983.

² Fouts, P. J., Helmer, O. M., and Lepkovsky, S., *PROC. SOC. EXP. BIOL. AND MED.*, 1939, **40**, 4; *Am. J. Med. Sc.*, 1940, **199**, 163.

³ Spies, T. C., Bean, W. B., and Ashe, W. F., *J. A. M. A.*, 1939, **112**, 2414.

⁴ Kark, R., Lozuer, E. L., and Meiklejohn, A. P., *PROC. SOC. EXP. BIOL. AND MED.*, 1940, **43**, 97.

solution was injected intracutaneously, congestion was evident for more than 4 days in 2 out of 3 animals.

Potency. In addition to physical and chemical characterization, crystalline vitamin B₆ may be assayed biologically. For this purpose, young rats of 21 to 23 days of age were fed a ration composed of sucrose 67%, vitamin-free casein 20%, McCollum's salt mixture No. 185 4%, Crisco 3%, liver filtrate free from B₆ 2%, cod liver oil 2%, and agar 2%. In addition, each animal received daily doses of 20 γ each of thiamin chloride and riboflavin. Towards the end of 6 to 8 weeks, these rats developed typical signs of B₆-avitaminosis—acrodynia and inhibition of growth. They were then treated individually with single doses of various size by mouth, and examined daily for 2 weeks. In a group of 60 rats, it was found that a dose of 40 to 60 γ cured acrodynia in an overwhelming majority of the rats within 14 days. Meanwhile, there was a decisive increase in their body weights. These results are comparable to those of Reedman, Sampson, and Unna.⁵ For testing the potency of a new lot of B₆ · HCl, it has been the practice of this laboratory to determine the median curative dose for acrodynia (CD₅₀), and compare it with the CD₅₀, determined simultaneously, of the purest lot which is being preserved as a standard.

Acute Toxicity. By intravenous injection, the median lethal dose (LD₅₀) of B₆ · HCl in mice was found to be 545.3 ± 42.9 mg per kg, and that in rats 657.5 ± 18.3 mg per kg. The data are shown in Table I. Tonic and then clonic convulsions occurred, and death followed rapidly. Animals either completely recovered or succumbed within 5 minutes after injection. No after effects were noted in the surviving rats. The acute toxicity of B₆ · HCl in rats

TABLE I.
Toxicity of Vitamin B₆ · HCl in Mice and Rats by Intravenous Injection.

Animal	Conc. of solution, %	Dose, mg per kg	No. died	LD ₅₀ \pm Standard error, mg per kg
			No. used	
Mice	1	300	0/5	545.3 ± 42.9
		400	1/5	
		500	1/5	
		600	3/5	
		700	3/3	
Rats	5	500	0/3	657.5 ± 18.3
		600	1/5	
		650	4/10	
		700	4/5	

⁵ Reedman, E. J., Sampson, W. L., and Unna, K., *PROC. SOC. EXP. BIOL. AND MED.*, 1940, **43**, 112.

by subcutaneous and oral administration has been estimated by Unna and Antopol.⁶

Repeated Administration. Five mice were injected by the tail vein with daily doses of 100 mg per kg except Saturdays and Sundays for 2 weeks. All of them gained weight during the course of medication. Upon sacrifice, no pathological changes were detected.

In Men. Twelve young male adults consented to take B₆·HCl by different routes as shown in Table II. The drug was dispensed in capsules for oral use, but made into 2.5% solution for injection purposes. Briefly, it may be stated that no ill effects were observed when B₆·HCl was given either by mouth or by intravenous injection in the dosage of 100 to 200 mg. Pain uniformly occurred when the drug was injected intramuscularly, perhaps due to its

TABLE II.
Action of Vitamin B₆·HCl in Men by Various Routes of Administration.

Subject No.	Age	Dose, mg			Reactions noted
		Oral	Intra-muscular	Intra-venous	
1	22	100	50	100	None "Sore" at site of injection for 1½ hours None
2	42	100			"
3	35	100			"
4	40	100			"
5	24	100	50	100	" "Aching" for 15 min None
6	33		50	100	" "Burning" for 15 min None
7	28		50	100	" "Sore" for 2 hrs None
8	30		50	100	" "Sore" for 15 min None
9	28		50	100	" "Aching" for 2 hrs None
10	21			200	"
11	29			200	"
12	21			200	"

⁶ Unna, K., and Antopol, W., PROC. SOC. EXP. BIOL. AND MED., 1940, **43**, 116.

acidity. In 3 out of 6 cases, it lasted as long as $1\frac{1}{2}$ to 2 hours. No other toxic manifestations were noted.

Other Effects. Casual observations were made on cats' blood pressure and respiration. Doses of 100 mg did not alter the pulse nor the respiratory rate; nor did they change the height of carotid pressure. Appropriate concentrations (1:8000) of $B_6 \cdot HCl$ caused brief inhibition of isolated rabbit's small intestines with prompt recovery, and contraction of the isolated guinea pig's uterus. The response here was not due to a low pH, because controls with the same acidity did not reproduce these results.

Summary. 1. Vitamin $B_6 \cdot HCl$ is acid in reaction which may be responsible for certain irritating effects in body tissues. 2. A method of bioassay has been described based upon the curing of rats' acrodynia. 3. The acute toxicity of $B_6 \cdot HCl$ in mice and rats by intravenous injection has been determined. 4. Mice can tolerate repeated doses of 100 mg per kg, given intravenously, without pathological

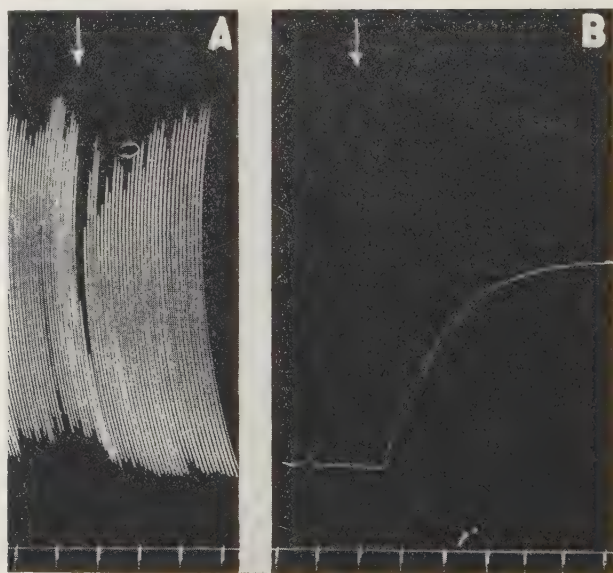


FIG. 1.

Action of Vitamin $B_6 \cdot HCl$ on Isolated Smooth Muscle Organs.

A. The peristaltic movements of a strip of a rabbit's small intestines immersed in Tyrode's solution at $38^\circ C$. At arrow, 0.5 cc of 2.5% solution of $B_6 \cdot HCl$ was applied (making the concentration 1:8000). There was a brief inhibition followed by prompt recovery.

B. The myogram of a horn of a virgin guinea pig's uterus immersed in Tyrode's solution at $38^\circ C$. At arrow, the same amount of $B_6 \cdot HCl$ as above was added. It resulted in a contraction.

changes. 5. No ill effects occur in men when $B_6 \cdot HCl$ is administered orally or intravenously in the dosage of 100 to 200 mg. Pain occurs when the drug is injected intramuscularly. 6. In the concentration of 1:8000, $B_6 \cdot HCl$ inhibits isolated rabbit's small intestines, but contracts the isolated guinea pig's uterus.

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Effects of Subcutaneous Injection of Estrogen upon Skeleton in Immature Mice.

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It is known that the subcutaneous administration of estrogen produces osteosclerosis in immature mice.¹ Hitherto, however, detailed roentgenographic and histologic studies explaining the mode of development of the osteosclerosis have been lacking. This paper reports such an investigation.

The study was conducted upon 66 immature mice, some of the C_3H strain and some from stock colony of the Rockland Farms. The experimental subjects received subcutaneous injections of estradiol benzoate in sesame oil* each week for a certain number of weeks. The control animals received injections of sesame oil for corresponding periods of time.

At the expiration of the total period allotted for the experiment, the animals were roentgenographed and autopsied. The skeletal tissues [femur, tibia, humerus, vertebral column, calvarium, ribs, pelvis, foot and jaw (including incisors)] were fixed in Helly's fluid, decalcified in 5% nitric acid, embedded in paraffin and stained with hematoxylin and eosin. The soft tissues were likewise prepared for histologic examination.

Table I summarizes the organization of the experiments.

The control mice received weekly injections of sesame oil.

Roentgenographic and Gross Pathologic Findings: Roentgenographic examination reveals that in immature mice osteosclerosis, caused by the formation of new bone in the medullary cavity, especially in the lower end of the femur and upper end of the tibia,

¹ Gardner, W. U., and Pfeiffer, C. A., *Proc. Soc. Exp. Biol. and Med.*, 1938, **37**, 678; *ibid.*, 1938, **38**, 599.

* Progynon-B was generously supplied by Schering Corporation.

TABLE I.

Series	Strain	No. of immature mice		Sex	Weekly dose of estrogen R.U.	No. of weeks treated
		Exp.	Control			
1	R. Farms	6	6	Female	1000	2-5
30	" "	6	6	Male	500	5-10
21	C ₅ H	7	7	Female	500	6-12
16	R. Farms	5	5	"	500	8-20
3	" "	3	3	"	300	4-14
2	" "	6	6	"	150	8-22

follows upon subcutaneous administrations of estradiol benzoate in sesame oil. When 1000 rat units of estrogen have been given for 3 or 4 successive weeks, an area of increased density is already observed in the sub-epiphyseal plate regions of the bones contributing to the knee joint. When the estradiol is given over the longer periods of time and in the larger doses, the dense shadow advances into, and may obliterate the medullary cavity. After administering 500 rat units of estradiol benzoate each week for 2 or 3 months, one notes an osteosclerosis of the distal third of the femur as well as of the proximal third of the tibia. This change occurs in both the male and female mice, but is somewhat less marked in the former.

The administration of 500 rat units of the estrogen per week for 4 or 5 months provokes a more generalized osteosclerosis. It may obliterate a considerable part of the medullary cavity and also cause a pronounced thickening of the cortices of all the long bones. The epiphyses, especially of the femur and tibia in the knee joint, are similarly transformed. The vertebral column also undergoes such changes. In the vertebrae, the sclerosis makes its first appearance in the zone of provisional calcification and then advances from the caudal and cephalad surfaces toward the middle of the vertebral body.

It is interesting that at no time were isolated foci of osteosclerosis observed. Furthermore, where cortical thickening is present, it seems to be the result of partial obliteration of the metaphyseal or diaphyseal medullary cavity. No periostitis is evident. The calvarium in general tends to become sclerotic, and usually does so after 3 or more months of administration of the estrogen in doses of 500 rat units per week. The density of the incisor teeth is somewhat increased. From some of the roentgenographs one gains the impression that the pulp spaces of the incisors and especially of the lower ones, are diminished in size.

Injections of 150 rat units of estradiol benzoate per week for 2 or 3 months, likewise causes a productive osteosclerosis, but to a lesser degree.

In general, the length of the skeleton, including that of the calvarium, is somewhat less in the experimental animals than in the controls.² This is especially true of the animals treated for 3 or more months.

Along with the rather general sclerotic changes and inhibition of growth, there is evidence of resorption in the pubic bones. This commences in the body of these bones and advances along the superior and inferior ramus. These transformations may be observed after 4 or more weekly injections of 500 rat units of estrogen. If such injections are continued for 8 weeks, the superior ramus of the pubic bone becomes very short, thin and dense. In many cases in which the administration of estrogen is continued for 12 or more weeks, the entire pubic bone undergoes resorption.

Histologic examination of the skeletal tissues of representative animals in each group reveals that the epiphysial plates—especially of the bones of the knee joint—are quickly affected. The columns of hypertrophic cells are diminished in height and their matrices show premature calcification. The activity of the resting and proliferating cells is likewise inhibited, in that their rate of division is retarded. The number of cartilage cells in the epiphysial plate is diminished to a slight degree. A striking finding is the absence of the columnar arrangement of newly formed trabeculae in the zone of provisional calcification. Instead of this arrangement, one finds a disorderly agglomeration of blood vessels which have erupted into the growth plate and which may have reached the level of the proliferating cells. Around the numerous blood vessels, new bone has formed by means of osteoblastic activity in the connective tissue in the region of the zone of provisional calcification.

The new trabeculae of bone contain very small amounts of cartilaginous matrix. Instead of growing in a normal strictly vertical plane, the sheets of new bone develop in a disorderly fashion; a good many are in a horizontal plane. Most of the trabeculae fuse to form an almost solid block of bone.

The new bone rapidly fills up the medullary cavity. It advances into the diaphysial marrow cavity by spreading along the endosteal surfaces of the cortices.

It is interesting that in a group of female mice (Series 3) new bone formation was associated with resorptive phenomena. Specifically, the vessel canals were found enlarged in the cortices of the long bones and in the tables of the calvarium. Furthermore, in many places, the linings of these canals showed occasional osteoclasts.

² Zondek, B., *Folia Clin. Orient*, 1937, **1**, 1.

The region of the zone of provisional calcification of the bones comprising the knee joint presented a very loose vascular osteogenic tissue in which areas of new bone were forming. The connective tissue spread for a considerable distance into the diaphysial portion of the marrow cavity. In the calvarium, the newly formed intramedullary connective tissue appeared to arise from the stroma of suture lines.

In the other series of animals, the bone-forming elements were predominant. In fact, the new bone may fill a large portion of the medullary cavity and replace a considerable amount of the lymphoid marrow. Although the most obvious effects occur in the metaphysial region of the bones of the knee joint, the upper end of the femur and the humerus, and the vertebral bodies become similarly involved, though to a lesser degree. Replacement of the lymphoid marrow by new bone occurs in the epiphyses also, and especially in those of the bones comprising the knee joint region. In these epiphyses, the subchondral areas become sclerotic.

In the articular cartilages, as in the growth plates, the cells are retarded in their activity. The cartilage cell outlines in the columnar regions are much more distinct than they would normally be. In the zone of provisional calcification, the blood vessels may advance into the calcified cartilage beyond the normal level. Interestingly enough, however, the matrix of the articular cartilage above the zone of provisional calcification does not undergo premature fibrillization or degeneration.

In general, the extent and severity of the changes in the bone and cartilage vary in accordance with the duration of the experiment and the dosage of estrogen. In the course of 4 to 5 months, the femur, tibia, and other long bones and the vertebral column, may come to show extensive replacement of the lymphoid elements in the metaphysial and diaphysial portions of the medullary cavity by newly formed bone. There is no increase in periosteal activity.

Conclusion. Injections of estradiol benzoate into immature female and male mice in the dosages listed in the table, stimulate the proliferation of new bone in the medullary cavities of certain bones and especially of the lower end of the femur and upper end of the tibia. Other bones, including the calvarium, are also affected, but to a lesser degree. The proliferation begins around the zone of provisional calcification and advances into the diaphysial portion of the bone. The changes in question can easily be recognized on roentgenographic examination. When the pubic bones are involved, they undergo in addition, more or less resorption, the extent of which depends upon the dosage of estrogen and the duration of the experiment.

Reversibility of Digitalis Action.*

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In Cushny's monograph on digitalis one reads the following regarding drugs of the digitalis group, "The action of these drugs is therefore reversible only in the earlier stages, and that slowly and with difficulty; later the process becomes irreversible, and the poison can be displaced neither during life nor by the use of chemical solvents after death."¹ Again, Straub,² speaking of the excised adult heart treated with digitalis, states "the final effect, after it has once set in, cannot be removed by any amount of washing. The reaction of the digitalis glucoside with the heart muscle is not reversible, and the heart is killed." This opinion is widespread.

In contrast to the idea of irreversibility of digitalis action, Kingisepp³ demonstrated the ability of electrically driven isolated frog ventricles to respond to digitalis wash-out. Arrested ventricles were made to return to their original amplitude of response as measured "by the isochoric response." In the present work further evidence as obtained from the study of the embryonic heart is given. After complete stoppage it has been possible to revive the hearts and return them to entire normality. Before presenting our data we wish to express a warm sense of appreciation for Doctor Torald Sollmann's kindly aid.

Method. Three drugs were used: ouabain in 1:100,000 dilution; tincture of digitalis in 1:100 dilution made from Reference Digitalis Powder according to U.S.P. XI; and digitoxin, as much as would dissolve in Tyrode solution in an hour's time. In each case the diluent was glucose-free Tyrode solution at pH 7.4.

The ventricle and conus of chick embryos (hereafter called ventricle), incubated for 48 hours, were excised and placed in a drop of Tyrode solution in a well slide. A coverslip was set over the well

* This research was made possible by a grant from the Council on Pharmacy and Chemistry of the American Medical Association and a grant from the John and Mary R. Markle Foundation.

¹ Cushny, A. R., *The Action and Uses in Medicine of Digitalis and Its Allies*, p. 80, Longmans, Green and Co., New York, 1925.

² Straub, W., *Stanford University Publications, Medical Sciences*, 1929, **3**, 45.

³ Kingisepp, G., *J. Pharm. and Exp. Therap.*, 1935, **55**, 377.

to prevent excessive evaporation and the preparation was placed in a warm chamber at 38°C. After the ventricle had reached incubator temperature the rate of beat was taken. Next, Tyrode solution around the ventricle was pipetted off and replaced by a drop of the desired glucoside diluted with Tyrode solution. After being completely stopped by the glucoside, the ventricle was removed with a platinum loop to a petri dish of Tyrode solution; then to a second dish; and finally removed to a well slide containing Tyrode solution, covered, and returned to the warm chamber. This constituted what we shall call a "wash". In some cases this procedure was repeated (digitalis, wash, digitalis, wash, etc.). Rates of beat, time required for stoppage in the glucoside, time of appearance of first beats after washing was started, time required for the return of normal beating, and other pertinent data were recorded.

Observations. For purposes of comparison 10 experiments with ouabain in 1:100,000 dilution in Tyrode solution were conducted. Straub⁴ demonstrated the reversibility of this glucoside in adult frog hearts. Paff and Johnson⁵ noted its reversibility in the embryonic heart. Table I summarizes the data. In the strength used the drug stops the ventricles completely in about 3.5 min (the median). The mean is really 4.6 min for the 10 experiments but discrepancies in preparations 8 and 10 raise the average stop time considerably. Here, as in all later experiments, this is partly due to the fact that the first rate of beat was taken with the ventricle in Tyrode solution and this was replaced by the glucoside. It was not

TABLE I.
Ventricles in 1:100,000 Ouabain.

No.	Rate per min	Min in drug	First beat after wash	Recovery	
				Time	Rate/min
1	103	2 + 3*	9 min	12 min	138
2	141	2 + 3	1	53	155
3	111	4 + 1	5	17	157
4	160	1 + 4	13	19	149
5	133	3 + 2	10	18	137
6	61	4 + 1	8	12	88 (irregular)
7	72	4 + 1	7	15	119
8	126	9 + 3	4	14	152
9	116	3 + 2	6	8	145
10	92	14 + 1	7	33	92
Mean:	112/min	4.6 + 2.1 min	7 min	20 min	133/min

*First number is time of complete stop. Second number is time left in drug after stop.

⁴ Straub, W., *Biochem. Z.*, 1910, **28**, 392.

⁵ Paff, G. H., and Johnson, J. R., *Am. J. Physiol.*, 1938, **122**, 753.

desirable to remove all the Tyrode solution for fear that the ventricle would be damaged. Even small amounts can produce sufficient variation in concentration to alter appreciably time of appearance of stoppage. The ventricles were left in the ouabain 2.1 min (mean) after complete stoppage. The time when first beats occurred after washing was started averaged 7 min. These first beats were not always regular in rate. In the table the time after washing when the beats did become regular and deep is indicated under the heading "Recovery". The rate of 6 and 7 after recovery are qualified by "(irregular)". Both of these showed periods of slight acceleration followed by periods of slower rate of beat. They are not significant since controls sometimes show the same thing.

The next group of experiments involved the use of tincture of digitalis in 1:100 dilution. Tincture was made from Reference Digitalis Powder (1 cc contained 1 U.S.P. XI unit). In Table II results are summarized. The average time of ventricular stoppage was 3.4 min. After stop the preparation was left in the diluted tincture 2.7 min on the average. The first beats after wash was started appeared in 15 min (average). A difference between tincture and ouabain was noticed here however. The first ouabain beats were usually of a nature involving the entire ventricle. In the tincture the first beats were usually slight "jiggles" near the end of the ventricle from which the atrium had been cut. These gradually increased in depth and involved more and more of the ventricle until the entire preparation was beating. It became obvious that the tincture was more difficult to remove than ouabain since irregularities usually persisted so long as to make a second wash advisable. The mean recovery time was 46 min and the average rate of beat was 134 per min. Ventricles 4 and 5 showed

TABLE II.
Ventricles in 1:100 Tincture Digitalis (U.S.P. XI).

No.	Rate per min	Min in drug	First beat after wash	Recovery	
				Time	Rate/min
1	123	2 + 3	22 min	36 min	164
2	114	2 + 3	16	55	158
3	89	4 + 5	26	50	132
4	118	2 + 3	12	25	130 (irregular)
5	69	2 + 3	27	55	130
6	73	4 + 1	2	70	179
7	121	5 + 2	19	22	165
8	80	3 + 2	4	50	122
9	129	3 + 2	5	50	107
10	82	7 + 3	16	45	56
Mean:	100/min	3.4 + 2.7 min	15 min	46 min	134/min

irregularities, but here again they were of a nature comparable to those seen in the controls.

The results with a single stoppage by tincture encouraged the attempt at repeated stoppage and washing with the same ventricles. The mere mechanical manipulation alone involved in this process offers a real hazard to continued activity of the embryonic ventricle. Proof that it can be successfully carried out is seen, however, in the following experiment:

Ventricle, rate, 90/min. Stopped in tincture in 2 min 40 sec. Washed 17 min. Beat again 94/min (irregular). Stop in tincture again in 45 sec. After 48 min wash the rate was 110/min (irregular). Stopped the third time in tincture in 1 min 15 sec. Washed 20 min, and beat became regular at 160/min. Stopped a fourth time in tincture in 1 min 3 sec. After 50 min of washing beat returned and was irregular. No rate was taken at this time. Stopped a fifth time in tincture in 55 sec. Wash 25 min and irregular beat returned. Again no rate taken. A sixth stop occurred in 47 min, and after recovery the rate was irregular. Mean rate was 22/min. Four other ventricles gave comparable results.

In the third group of experiments digitoxin was used. The same technic applied successfully with tincture and ouabain failed in 4 out of 5 attempts with digitoxin even 150 min after the single wash had begun. It was necessary to change the technic. This consisted in repeated washes at intervals of 15 min until it was apparent that complete recovery would occur. Results are summarized in Table III.

Discussion and Further Observations. The tabulated results indicate that both digitoxin and tincture as well as ouabain can be washed out of the embryonic ventricles. Of the 3, digitoxin is the most difficult to remove. Tincture is next and, as was expected, ouabain was removed with ease. The short time in which the ventricles remained in the glucosides raises the question whether or not the drug had sufficient time to act. As proof that the time is ample it was noted that mechanical manipulation of the ventricles invariably

TABLE III.
Ventricles in Digitoxin.

No.	Rate per min	Min in drug	No. of washes (15-min intervals)	First beat after wash	Recovery	
					Time	Rate/min
1	104	5 + 2	8	64 min	142 min	111
2	99	9 + 2	6	80	85	133
3	90	13 + 2	7	82	92	115
4	116	2 + 3	6	56	119	90
Mean:	102/min	7.2 + 2.2 min	6.7	71	110	112/min

failed to elicit a response. More conclusive evidence is the fact that controls stopped by digitoxin, for example, remained absolutely quiescent to single make and break induction shocks. Furthermore the ventricles, when removed from the glucosides, continued to be affected as evidenced by the fact that they passed into extreme systole. This is especially true with tincture and digitoxin. The picture at this stage was that of a dead ventricle. Accompanying this reaction it was noted that the mesothelium at the surface of the heart seemed to become swollen beyond the amount explainable by the systole. The cells appeared rounded. We believe it also significant that the ventricles became sticky, so much so that they often adhered strongly to the glass or to the platinum loop used in transfer. When this last occurred, small portions of the heart were sometimes torn away, suggesting that the process seriously involved the muscle as well as the mesothelium.

These stages suggest marked degenerative changes. However, despite this suggestion, they are only apparent. As proof of this, ventricles stopped in tincture and in digitoxin were placed in chicken blood plasma in Carrell flasks along with controls. The results were conclusively in favor of removal. Not only did all the ventricles, both controls and glucoside-treated hearts (after recovery), beat for an observation period of 3 days but the beats, both as to rate and depth, were normal. Unless one knew which ventricles had been treated with the glucosides, it would be impossible to distinguish drugged hearts from controls. This is equally true of both physiological activity and structural integrity.

In the embryonic heart additional evidence is necessary before a reasonable hypothesis regarding the probable nature of digitalis action can be stated. The production of stickiness and rounded appearance of cells suggests an exchange of material between cell and environment accompanied by marked surface phenomena. It can be postulated, however, that no firm chemical union occurs between the digitalis and the embryonic heart muscle since the time necessary for recovery is so short.

Summary and Conclusions. 1. In embryonic ventricles stopped by ouabain, tincture of digitalis, and digitoxin the effects of the drugs can be removed by washing with Tyrode solution. 2. Of the 3 glucosides, the action of digitoxin is the most difficult of removal. Tincture, in turn, is more difficult to remove than ouabain. 3. With tincture, the process of stop, wash-out, stop, wash-out, etc., can be repeated at least 6 times in the same ventricle. 4. Digitoxin-stopped ventricles recover in blood plasma and are indistinguishable as to function and structure from controls in the same medium.

Infection of the Developing Chick Embryo with Dysentery Bacilli.

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The experimental approach to the problems connected with micro-organisms of the *Shigella* group is greatly hampered by the lack of a convenient laboratory animal sensitive to this type of infection. The purpose of this paper is to report on our experiences with the infection of chick embryos with dysentery bacilli, which we hope may contribute toward the alleviation of this difficulty.

Goodpasture and his coworkers have studied bacterial infection in the chick embryo and have investigated the reactions of this new host.^{1, 2, 3} There are also some reports from other laboratories on applications of this method to problems of a similar scope.⁴⁻⁷

For our study we have used 2 strains of *Shigella dysenteriae* ("Shiga bacillus"), 7 of *Shigella paradysenteriae* ("Flexner bacillus"), and 7 of *Shigella sonnei*. All were carefully controlled for their microscopic, cultural and serological properties.

We found fertile eggs incubated 9 to 10 days most favorable for our purposes. We followed, in the main, Goodpasture's procedure.^{8, 9} If the usual precautions of bacteriological work are applied, the danger of contamination of the eggs is certainly not greater than that of agar plates.

The number of viable microorganisms introduced was checked by plate count of 10^{-7} dilutions of the broth culture. On the average the broth cultures contained 500,000,000 microorganisms per ml. The eggs were infected by dropping 0.1 ml amounts of broth diluted serially 10-fold with saline solution upon the chorio-allantoic membrane. The infected eggs were incubated at 37.5°C.

¹ Goodpasture, E. W., and Anderson, K., *Am. J. Path.*, 1937, **13**, 149.

² Goodpasture, E. W., *Am. J. Hyg.*, 1938, **28**, 111.

³ Buddingh, G. J., and Polk, A. D., *J. Exp. Med.*, 1939, **70**, 485, 489, 511.

⁴ Morrow, G., and Berry, G. P., *J. Bact.*, 1938, **38**, 38.

⁵ Morrow, G., Syverton, J. T., Stiles, W. W., and Berry, G. P., *Science*, 1938, **88**, 385.

⁶ Moore, M., *Science*, 1939, **89**, 1939.

⁷ Sterzi, G., and Staudacher, V., *Giorn. ital. di dermat. e sif.*, 1939, **17**, 4.

⁸ Goodpasture, E. W., and Buddingh, G. J., *Am. J. Hyg.*, 1935, **24**, 319.

⁹ Burnet, F. M., *Med. Res. Council, Spec. Rep. Ser.* 220, London, 1936.

All 3 types of *Shigella* mentioned above grew very well on the egg membrane. From one to 10 microorganisms were found to be enough to obtain growth on the membrane. If the growth was only scarce, no gross changes were seen on the membrane. If infection was intense, infiltrates varying from small greyish spots to purulent exudates, sometimes also small hemorrhages, were seen. The degree of this reaction was roughly proportional to the amount of bacteria implanted.

Generalized infection could be produced with each strain. The amounts necessary for this purpose varied from 50 to 50,000 microorganisms. They were fairly constant for each strain. Where generalized infection took place, the microorganisms could be recovered from the heart blood and the organs of the embryo, and the embryos died in 24 to 72 hours, exceptionally also on the fourth day after infection.

Smooth and partially rough cultures were infective to the same degree. However, perfectly rough variants were found to be devoid of invasive power, and thus did not kill the embryos. With our Flexner strains, there were several observations of the reversion from partially rough to smooth after egg passage. No such reversion was observed with Sonne strains.

In many cases quick disintegration of the contents of the eggs followed death. This was especially marked in cases infected with Sonne strains. Where the dead embryos were well preserved, no characteristic findings were noted upon inspection.

We are planning a histological study of both membranes and embryos in order to obtain information on the host's reaction and on possible special localizations of the infective agent.

It was found also possible to cause a lethal infection by injection of dysentery bacilli into the yolk sac of the embryo. In this case, generalized infection takes place within 6 hours.

Bacteriostatic Effects of Sulfanilamide, Pyridine and Thiazol Derivatives upon Colon-Typhoid-Dysentery Group.*

C. A. LAWRENCE. (Introduced by O. W. Barlow.)

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Chemotherapeutic studies of a series of thiazol derivatives of sulfanilamide under conditions of experimental infections in animals with *beta* hemolytic streptococci (strain C203), *Staphylococcus aureus*, and pneumococci Types I, II and III as reported by Fosbinder and Walter,¹ McKee, *et al.*,² and Barlow and Homburger^{3, 4} suggested that at least 2 of these compounds merited careful clinical examination. A comparison of the *in vitro* effects of these compounds on the above organisms indicated that they were superior to either sulfanilamide or sulfapyridine (Lawrence⁵).

The present investigation was undertaken to determine the comparative *in vitro* effects of the thiazol compounds (sulfathiazol, sulfamethylthiazol and sulfaphenylthiazol) with those of sulfanilamide and sulfapyridine upon additional groups of organisms, namely, the gram negative bacilli representative of the colon-typhoid-dysentery group. These included 5 strains of *Eberthella typhosa*, one culture of which had recently been isolated from a typhoid patient (No. 1006), 2 cultures each of *Escherichia coli* and *Aerobacter aerogenes*, and one strain each of *Salmonella paratyphi*, *S. schottmuelleri*, *S. suispestifer*, *S. psittacosis*, *S. enteritidis*, *Shigella dysenteriae* and *Proteus vulgaris*.

Method. Ten mg % drug-broth solutions were prepared by adding the dry powders to 100 cc quantities of veal dextrose broth of pH 7.4 and containing bacto peptone.† Since preliminary cultural

* The author wishes to express his appreciation to Dr. J. J. Clemmer for many of the cultures used in this study.

¹ Fosbinder, R. F., and Walter, L. A., *J. Am. Chem. Soc.*, 1939, **61**, 2033.

² McKee, G. M., Rake, G., Greep, R. O., and Van Dyke, H. B., *PROC. SOC. EXP. BIOL. AND MED.*, 1939, **42**, 417.

³ Barlow, O. W., and Homburger, E., *PROC. SOC. EXP. BIOL. AND MED.*, 1939, **42**, 792.

⁴ Barlow, O. W., and Homburger, E., *PROC. SOC. EXP. BIOL. AND MED.*, 1940, **43**, 317.

⁵ Lawrence, C. A., *PROC. SOC. EXP. BIOL. AND MED.*, 1940, **43**, 92.

† A few crystals remaining at the bottom of the flask containing the sulfaphenylbroth solution, after autoclaving and cooling to 37°C, indicated this solution to be slightly supersaturated.

studies indicated that the particular strain of dysentery organisms used in this investigation grew more luxuriantly in a dextrose-free veal medium, the carbohydrate was omitted from the broth in which this organism was studied. The medicated culture media were autoclaved at 10 lb for 10 minutes and upon cooling 1 cc of an 18-hour broth diluted culture of one of the several organisms was added to each drug-broth and drug-free control broth medium.

The inoculated solutions were incubated at 37°C for 23-25 hours. In order to estimate the degree of bacteriostasis at this time, the following procedures were carried out. One-tenth and 1.0 cc of appropriate broth dilutions were placed in each of 2 petri dishes and melted and cooled (45°C) veal dextrose agar added. The contents were mixed thoroughly by swirling and the agar allowed to solidify. The plates were incubated at 37°C for 72 hours at which time the growing colonies were counted.

Results. In Table I are presented the results of the *in vitro* effects

TABLE I.
Comparison of *In Vitro* Effects of the Several Compounds upon *E. typhosa*, *E. coli*,
and *S. dysenteriae*.

10 mg% concentrations. 23-25 hrs. 37°C. Organisms/cc.							
Organism	Inoculum Bacteria/cc	Sulfanil- amide	Sulfa- pyridine	Sulfa- thiazol	Sulfa- methyl- thiazol	Sulfa- phenyl- thiazol	Control
<i>E. typhosa</i>	45	420 M	125,000	35,000	20,000	—	570 M
“Rawling” “B”	75	330 M	450	0	250	320,000	670 M
<i>E. typhosa</i>	40	500 M	780 M	110 M	100 M	610 M	660 M
“Rawling” “M”	80	725 M	490 M	120 M	40 M	410 M	590 M
<i>E. typhosa</i>	40	520 M	670 M	340 M	350 M	530 M	600 M
“Hopkins”	60	450 M	660 M	40 M	135 M	370 M	490 M
<i>E. typhosa</i>	70	620 M	460 M	370 M	410 M	640 M	930 M
No. 305	60	630 M	375 M	215 M	170 M	380 M	650 M
<i>E. typhosa</i>	70	620 M	750 M	370 M	440 M	580 M	830 M
No. 1006	60	750 M	620 M	230 M	290 M	620 M	890 M
<i>E. coli</i>	17	368 M	28 M	3 M	6 M	46 M	754 M
“B”	90	740 M	1 M	490,000	720,000	250,000	1.1 B
<i>E. coli</i>	23	1.1 B	112 M	3.3 M	4.7 M	32 M	1.2 B
“M”	75	1 B	40 M	4 M	17 M	86 M	1.2 B
<i>S. dysenteriae</i>	4	484 M	308 M	130 M	122 M	145 M	425 M
	14	595 M	342 M	112 M	144 M	122 M	518 M

— = Compound not tested.

0 = No growth in plate inoculated with 0.1 cc. of the undiluted drug broth solution or dilutions thereof.

M = Million.

B = Billion.

of the several compounds upon *E. typhosa*, *E. coli* and *S. dysenteriae*. While the differences in the degree of bacteriostasis produced by most of these compounds under these experimental conditions are not striking, there is a definite trend which indicates a greater inhibitory effect on the part of sulfathiazol and sulfamethylthiazol than that of the other compounds.

A comparison of the effects of the compounds upon the *Salmonella*, *Aerobacter* and *Proteus vulgaris* organisms is given in Table II. In general sulfathiazol and sulfamethylthiazol were again found to be somewhat more effective than sulfapyridine, and distinctly more active than sulfaphenylthiazol and sulfanilamide in their bacteriostatic actions upon the organisms studied.

These findings, in part, confirm the results obtained by Helmholtz⁶ who, under different experimental conditions, was able to show that sulfathiazol and sulfamethylthiazol were more effective than sulfanilamide and sulfapyridine in their *in vitro* actions upon many of the organisms associated with urinary infections.

TABLE II.
Comparison of the *In Vitro* Effects of the Several Compounds upon *Salmonella*, *Aerobacter* and *Proteus vulgaris* Organisms.

10 mg% concentrations. 23-25 hrs. 37°C. Organisms/cc.							
Organism	Inoculum Bacteria/cc	Sulfanil- amide	Sulfa- pyridine	Sulfa- thiazol	Sulfa- methyl- thiazol	Sulfa- phenyl- thiazol	Control
<i>S. paratyphi</i>	110	1.3 B*	520 M	770,000	7 M	810 M	1.1 B
	155	1 B	600 M	260 M	225 M	520 M	1.1 B
<i>S. schottmuelleri</i>	160	1.6 B	730 M	8 M	115 M	860 M	1.6 B
	185	1.5 B	650 M	460 M	690 M	1.2 B	2 B
<i>S. suispestifer</i>	95	180 M	1,200	150	400	1,150	890 M
	100	340 M	80	40	270	10	740 M
<i>S. psittacosis</i>	70	380 M	350 M	15 M	60 M	700 M	580 M
	75	610 M	278 M	67 M	195 M	500 M	725 M
<i>S. enteritidis</i>	120	1 B	37 M	270,000	600,000	680 M	1.2 B
	47	690 M	630 M	50 M	105 M	400 M	720 M
<i>A. aerogenes</i> "B"	17	446 M	286 M	120 M	132 M	355 M	512 M
	26	425 M	224 M	94 M	160 M	376 M	489 M
<i>A. aerogenes</i> "M"	90	160 M	74 M	35 M	78 M	83 M	940 M
	150	1.4 B	430 M	60 M	90 M	900 M	1.2 B
<i>Proteus vulgaris</i>	37	460 M	13 M	1.5 M	1.5 M	15 M	290 M
	5	472 M	220 M	151 M	135 M	22 M	460 M

*See legend under Table I.

⁶ Helmholtz, H. F., *Proc. Staff Meetings, Mayo Clinic*, 1940, **15**, 65.

In a more recent publication Long and Bliss⁷ also found that the unsubstituted thiazol derivative was equal to, or slightly superior to, sulfanilamide and to sulfapyridine in its bacteriostatic action upon cultures of *E. coli* and *B. proteus* in broth.

Conclusions. On the basis of *in vitro* studies sulfathiazol and sulfamethylthiazol have been found to be somewhat more effective than sulfapyridine, sulfaphenylthiazol and sulfanilamide in their *in vitro* effects upon bacteria representative of the colon-typhoid-dysentery group. In general, the unsubstituted thiazol derivative appears to be the most active compound, being followed in decreasing order of effectiveness by sulfamethylthiazol, sulfapyridine, sulfaphenyl thiazol and sulfanilamide.

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Changes Produced by Desoxycorticosterone Overdosage in the Rat.*

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Kuhlmann, *et al.*,¹ found recently that dogs chronically treated with very high doses of desoxycorticosterone acetate show definite signs of damage and reveal blood chemical changes which appear to be the opposite of what is seen in adrenal insufficiency. Thus they noted “. . . . a striking decrease in serum potassium, a slight increase in serum sodium, a slight decrease in serum protein and non-protein nitrogen”. In this connection it appears of interest to mention our experiments in the rat which indicate that treatment with as high a dose as 10 mg of desoxycorticosterone acetate daily given for 20 days fails to produce any externally visible signs of damage and that, contrary to expectations, the blood chlorides prove to be consistently low. Meanwhile we have no explanation for the fact that although desoxycorticosterone prevents hypochloremia in the adrenalectomized rat, chronic overdosage with this

⁷ Long, P. H., and Bliss, E. A., *PROC. SOC. EXP. BIOL. AND MED.*, 1940, **43**, 324.

* The expenses of this investigation have been defrayed in part from a grant in aid received from the Schering Corporation of Bloomfield, N. J. The desoxycorticosterone acetate used in our experiments has been kindly supplied by Drs. G. Stragnell and E. Schwenk of the Schering Corporation.

¹ Kuhlmann, D., Ragan, C., Ferrebee, J. W., Atchley, D. W., and Loeb, R. F., *Science*, 1939, **90**, 496.

TABLE I.
Changes in Blood Chemistry and Adrenal Weight Induced by Desoxycorticosterone Overdosage.

Treatment	No. and sex of animals	Body wt	Blood chloride	Blood glucose	Adrenal wt
Desoxycorticosterone acetate 2 mg in 0.1 cc oil/day for 20 days	6 ♂ 6 ♀	157 (124-185) 100 (74-135)	380 (338-396) 335 (326-349)	99 (92-107) 86 (71-103)	40 (26-58) 36 (30-52)
Oil controls 0.1 cc/day for 20 days	6 ♂ 6 ♀	159 (142-172) 108 (73-145)	429 (425-431) 430 (420-443)	95 (83-103) 95 (88-107)	35 (29-44) 42 (38-53)
Desoxycorticosterone acetate 10 mg in 0.4 cc oil/day for 20 days	6 ♂ 6 ♀	159 (147-174) 122 (103-131)	380 (369-404) 381 (374-410)	70 (67-79) 86 (75-96)	19 (15-21) 22 (19-28)
Oil controls 0.4 cc oil/day for 20 days	6 ♂ 6 ♀	167 (147-178) 116 (106-131)	430 (421-456) 447 (438-456)	82 (75-92) 78 (71-92)	38 (33-43) 53 (48-65)
Desoxycorticosterone acetate 10 mg in 0.4 cc oil/day for 20 days	6 ♂	150 (140-159)	334 (322-351)	85 (71-96)	20 (17-22)
Cholesterol controls 10 mg in 0.4 cc oil/day for 20 days	6 ♂	150 (140-160)	400 (380-416)	86 (71-96)	40 (35-43)

compound decreases the blood chloride concentration in normal animals. Our experiments clearly indicate, however, that this is the case. The blood chlorides were determined by the Van Slyke method and are expressed in mg of NaCl/100 cc of blood. Blood sugars (Hartmann-Shafer-Somogyi's method) are expressed in mg of glucose/100 cc and the adrenal weight in mg. White Wistar rats were used for all experiments. Desoxycorticosterone was administered once daily by subcutaneous injections in peanut oil, the last injection being given 24 hours before autopsy. All animals were fasted for 24 hours before the determinations. In Table I, which summarizes our findings, the average values are given with the range of variations in brackets.

As the table indicates, the blood chlorides are significantly decreased in each case while the blood sugar does not appear to be affected. The absence of hypoglycemia indicates that overdosage with desoxycorticosterone acetate does not elicit all the symptoms of adrenal insufficiency. Gross estimations of the blood volume of our animals show that a decrease in the amount of the circulating blood—also characteristic of adrenal deprivation—likewise fails to occur in case of desoxycorticosterone overdosage. We mention this particularly because, contrary to the statement of Kendall,² who claimed that desoxycorticosterone and its acetate cause no significant adrenal atrophy—and thereby differ from corticosterone and compound E—we noted in agreement with our previous findings³ that the adrenals became very atrophic. It should be stated, however, that Kendall used relatively small doses of desoxycorticosterone and that from his findings, it appears that corticosterone and his compound E are even more active in causing adrenal involution.

Summary. Experiments on the rat indicate that chronic treatment with desoxycorticosterone acetate in daily doses of up to 10 mg does not lead to any significant external signs of damage but causes marked hypochloremia and adrenal atrophy. Since the blood sugar and blood volume is not significantly influenced by this treatment, the hypochloremia cannot merely be regarded as a sign of general adrenal insufficiency resulting from the atrophy of the adrenal cortex unless one assumes that the compound interferes specifically with the chloride regulating function of these glands. It should be emphasized, however, that even such doses of desoxycorticosterone acetate which do not suffice to cause significant adrenal atrophy produce definite hypochloremia.

² Kendall, Edward C., *Proc. Am. Soc. Biol. Chem.*, New Orleans, 1940.

³ Selye, Hans, *Canad. Med. Assn. J.*, 1940, **42**, 113.

Effect of Small Doses of Testosterone Propionate on the Testis.*

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Practically all reports concerning the effect of testosterone propionate on the testis stress the depressing effect of this hormone.^{1, 2, 3} Biddolph,⁴ using doses as small as 2 γ daily in animals treated from birth has, however, reported that the testes of his treated animals were practically unaffected. Since this author did not simultaneously publish any data concerning the accessory sex organs of his treated group it is conceivable that the dose which he used was so small that it not only failed to affect the testes but was also devoid of any androgenic effect. For this reason the following study was undertaken.

Thirty-six male albino rats (*Mus norvegicus*, var. *albus*) of Wistar Institute strain were divided into 2 groups consisting of 16 test animals and 20 littermate brother controls respectively. The test animals were given daily subcutaneous injections of 10 γ of testosterone propionate in sesame oil (Perandren) for 10 days beginning on the 22nd day of life. Except for these injections all animals were similarly treated, being fed on a diet of Purina dog chow daily and green vegetables twice weekly. Water was constantly present. The controls received no injections, since the solvent used (sesame oil) has been shown to have no sex-stimulating qualities.⁵ The animals were weighed before the first injection and at the time of sacrifice which was carried out by carotid incision under ether anesthesia at 32 days of age. Testes devoid of epididymides and seminal vesicles were excised, weighed, fixed in Bouin's solution and stained by haemotoxylin and eosin for microscopic study. The epididymides were excised and similarly prepared for microscopic study. This was done because it had previously been noted that the epididymis through its tubular contents

* The authors appreciate the aid of the Ciba Pharmaceutical Products Company, Inc., for partially defraying the expenses of this study and for supplying the testosterone propionate (Perandren) used.

¹ Moore, C. L., and Price, D., *Anat. Rec.*, 1938, **71**, 59.

² Korenchevsky, V., and Hall, K., *Brit. Med. J.*, 1939, **1**, 4.

³ Korenchevsky, V., Dennison, M., and Hall, K., *Biochem. J.*, 1937, **31**, 1434.

⁴ Biddolph, C., *Anat. Rec.*, 1939, **73**, 447.

⁵ Stone, C. P., *J. Comp. Psychol.*, 1938, **25**, 445.

may serve as an index of testicular activity.⁶ The data for each group were massed and analyzed statistically by Fisher's method.⁷ A *P* less than 0.05 was held as the criterion for probable significance of any difference observed between the test and control groups.

Results. Neither initial nor final body weights of the test group (32.1 g and 73.8 g respectively) showed any significant difference from the corresponding control weights (30.9 g and 70.8 g respectively).

The seminal vesicles of the treated animals averaged 33.5 mg and were significantly heavier than the corresponding mean of 22.5 mg for the controls (*P* was less than 0.01). The weight of the testes of the treated group averaged 546 mg as compared to a mean of 563 mg for the controls. The difference of 17 mg was only a 3% difference and was statistically insignificant (*P* was greater than 0.1).

Microscopically, the testes of all animals were normal. Spermatogenesis was incomplete in both treated and control groups. The epididymides of the treated group, however, contained numerous actively mitotic immature spermatocytes within their tubules. In the normals, this was only an occasional finding.

Discussion. From this study it may be seen that an androgenically potent dose of testosterone propionate as judged by its stimulating effect upon the seminal vesicles, has spared the testis the depression heretofore described. Biddolph⁴ has also observed a practically negligible depression with 2 γ doses administered daily from birth to 31 days of age. From his report, however, one was at a loss to know whether his dosage was androgenically potent otherwise. The presence of immature spermatozoal forms in the tubules of the epididymis indicates that testosterone propionate stimulates proliferation of the germinal epithelium without hastening maturation. In these respects it acts like the gonadotropic hormones.⁶ It differs from the gonadotropins in that it fails to stimulate the interstitial tissue.

Conclusions. Testosterone propionate injected subcutaneously in 10 γ doses daily for 10 days to albino rats from 22 to 32 days proved to be androgenically potent causing a "probably significant" enlargement of the seminal vesicles. The testes, however, were neither depressed in weight nor were their histological pictures altered. Proliferation of the germinal epithelium was hastened.

⁶ Rubinstein, H. S., *Endocrinology*, 1938, **23**, 171.

⁷ Fisher, R. A., *Statistical Methods for Research Workers*, Oliver and Boyd, London, 1936.

An Effective Method of Intraneural Inoculation of Poliomyelitis Virus.*

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The uncertainty of results obtained by most investigators with intraneural injections of poliomyelitis virus has limited the use of this method of inoculation for inducing experimental poliomyelitis. Nevertheless, if the virus of poliomyelitis is truly neurotropic, one would expect that intraneural inoculation would be as effective as intracerebral inoculation, and simpler, if the virus could actually be made to come into contact with numerous nerve fibers, rather than being forced along and between connective tissue sheaths within the peripheral nerve. This of course is strongly suggested by the work of Fairbrother and Hurst,¹ who showed that trauma during intraneural injection facilitates "takes" by this method of inoculation.

Going one step farther, and with the knowledge that during the first few days after nerve section the nerve cells with axons cut are more susceptible to the virus than normal cells,² it was decided to determine whether simple section of a peripheral nerve and immersion of the central stump in virus suspension for a few minutes was sufficient to produce poliomyelitis. This method, which involves no mechanical injection pressure, and which places the virus in contact with the protoplasm of every axon in the nerve, was found to be highly successful in producing poliomyelitis. When a large nerve, such as the sciatic nerve, was used, this method of inoculation was invariably successful with two strains of known potency, the MV and Wfd³ strains.

In 9 Rhesus monkeys the sciatic nerve was sectioned with sharp scissors peripheral to the sciatic notch or at the mid-thigh level, and the central cut end then soaked in as little as 0.1 cc of 20% virus suspension for several minutes. Poliomyelitis resulted after an incubation period of 4-6 days. In 5 cases the leg on the side of inoculation was completely paralyzed on the fifth day; the opposite leg was usually paralyzed completely also during the course of the same day.

* This work was supported by a grant from the Commonwealth Fund.

¹ Fairbrother, R. W., and Hurst, E. W., *J. Path. and Bact.*, 1930, **33**, 17.

² Howe, H. A., and Bodian, D., *Proc. Soc. Exp. Biol. and Med.*, 1939, **42**, 346.

³ Trask, J. D., and Paul, J. R., *J. Bact.*, 1936, **31**, 527.

A definite quantitative factor was observed with respect to the number of nerve fibers exposed to the virus. In 3 cases in which only the nerve to the hamstring muscles was cut and the cut end then soaked with virus, no paralysis resulted. Inoculation of the cervical sympathetic trunk by the same method in 2 animals failed also. In one case in which the central cut end of the vagus nerve was immersed in virus for 3 minutes, paralysis did not result, whereas in another animal in which the vagus nerve was similarly soaked for 15 minutes, neck paralysis and hoarseness resulted after an incubation period of 17 days. Inoculation of the hypoglossal nerve by this method was also successful in one case, the incubation period being 4 days. Apparently then, the numbers of nerve fibers exposed, and perhaps the size of the fibers and the length of time immersed are significant quantitative factors. In any case, only a small quantity of virus suspension is necessary.

The significance and advantages of the above described method of inoculation of peripheral nerves are obvious. When sufficient numbers of axonal processes of nerve cells are exposed to small amounts of virus at a definite point in the peripheral nerve, paralysis results. Because of the definitely known location at which the virus first comes into contact with the nerve fibers, because of the assurance that all of the fibers of the nerve are placed in contact with the virus, and because in any particular nerve the numbers and sizes of fibers thus exposed can be determined, this method offers possibilities for quantitative determinations of virus potency, of speeds of transmission of viruses along nerves, and of other important but not easily obtained data.

Effect of Tyrosinase on Blood Pressure of Hypertensive Rats.

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Recent studies on the action of pressor substances in experimental arterial hypertension make it probable that some pressor material liberated by diseased kidneys is responsible for the elevation of blood pressure. Because this pressor substance may be a relatively simple amine, an attempt has been made to learn the action of enzymes capable of altering certain amines, upon the blood pressure of hypertensive and of normal animals. Rats were made hypertensive (1) by partial constriction of one renal artery, (2) by unilateral hydronephrosis, and (3) by unilateral renal injury, the other kidney remaining healthy. Tyrosinase, obtained from Professor J. M. Nelson of Columbia University, was injected intravenously into 37 animals. Blood pressure was measured by a Hamilton manometer, the needle of which was inserted into the femoral artery, and records made for 40 to 60 minutes thereafter. In a few instances another measurement of blood pressure was made several days later.

In every case the diastolic pressure of the *hypertensive* animals fell 30 mm Hg or more (Table I), and in no case did it return to the initial level. Indeed in only one animal (Rat G 104) did it subse-

TABLE I (Abnormal Animals).
Diastolic Blood Pressure mm Hg. Before and After the Injection of Tyrosinase.

Rat No.	Dose, cc	Control	15 min after injection	30 min after injection	Change at 30 min	Subsequent level
H 93	0.5	120	50	70	-50	85—24 hr later
H 94	1.0	110	81	65	-45	82—70 min "
H 95	0.4	102	50	65	-37	70—2 wks "
H 97	1.0	126	93	85	-41	
H 100	0.5	112	52	82	-30	60—5 days "
G 104	0.8	134	86	100	-34	92—2 wks "
G 113	0.5	110	65	69	-41	82—98 min "
G 115	1.0	120	102	74	-46	
G 117	0.8	132	86	92	-40	82—75 " "
G 121	0.5	110	104	80	-30	72—80 " "
G 122	0.5	110	100	72	-38	62—75 " "
G 123	0.6	128	90	88	-40	100—17 days "
G 140	1.0	128	75	68	-60	85—75 min "
I 19	0.75	112	60	60	-52	
I 22	0.4	138	108	96	-42	80—70 " "
		M = 120	M = 80	M = 78	M = -42	
					$\sigma = 7.8$	

quently rise above 100 mm Hg, falling later to lower levels; in 2 others it rose for a short time above 90. Changes comparable in magnitude occurred in the systolic pressure. No effect was noticed until the injection had been given 5 to 15 minutes, when a spontaneous fall was seen. The blood pressure of five rats followed 1, 5 and 14 days remained low. An inactive preparation of the enzyme gave no effect.

Variable results were seen in *normal* rats. In 2, the diastolic pressure fell significantly. In the remainder the change was of smaller magnitude or a distinct rise was noticed (Table II).

Five animals operated upon failed to develop hypertension, and injection of the enzyme into them gave inconsistent results similar to those found in normal rats.

Injection of the enzyme appeared to have no toxic effect upon the animals.

The fact that this enzyme acts consistently as a depressor in hypertensive rats and has a variable effect in normal ones suggests that some substance common to the former is changed. It is possible, from the specificity of the enzyme for phenolic configurations, that the substance contains one or more of these chemical groups. No adequate interpretation of these results can, however, be made now,

TABLE II (Normal Animals).

Diastolic Blood Pressure mm Hg. Before and After the Injection of Tyrosinase.

Rat No.	Dose, cc	Control	15 min after injection	30 min after injection	Change at 30 min	Subsequent level
22	0.5	90	121	110	+20	102—45 min later
23	0.5	60	70	60	0	60—61 " "
24	0.5	75	70	79	+ 4	79—64 " "
26	0.8	115	95	80	—35	
27	0.5	100	100	100	0	
28	0.75	70	50	52	—18	55—55 " "
29	0.7	94	70	60	—34	
31	1.0	114	100	100	—14	
32	0.8	85	118	114	+29	
33	0.5	108	110	87	—21	112—17 days "
34	0.5	80	86	92	+12	106—9 " "
35	0.5	105	126	120	+15	110—60 min later
36	0.5	100	112	112	+12	120—45 " "
37	0.5	136	144	136	0	
40	0.5	80	65	65	—15	
41	0.5	122	126	122	0	
42	0.5	102	104	98	— 4	98—45 " "
43	0.5	102	110	114	+12	110—60 " "
45	0.5	85	86	82	— 3	
80	1.0	80	65	87	+ 7	
		M = 95	M = 96	M = 93	M = —2	
					$\sigma = 52.4$	

nor can consideration of these effects be applied to any animals save rats with unilateral renal disease until further studies, now in progress, indicate that similar action upon blood pressure takes place on the use of such enzymes in other mammals.

Summary. The injection of tyrosinase into hypertensive rats consistently and markedly lowered the blood pressure, this effect appearing 5 to 15 minutes after intravenous administration. The use of this material in normal animals gave variable results.

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Effect of Two Steroid Compounds on Weight of Thymus of Adrenalectomized Rats.*

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The thymus gland of rats can be made to regress rapidly by the administration of extracts of the adrenal cortex or by the administration of some of the steroid compounds occurring in the extracts. In studies of the biologic effects of 11-desoxy-corticosterone acetate and 17-hydroxy-11-dehydro-corticosterone acetate it was noted that the latter substance was the more active of the two in producing thymus atrophy.

Male rats of the Sprague-Dawley strain each having an initial body-weight of approximately 180 g were used. The diet was Purina Dog Chow. The test substances were dissolved in sesame oil and administered twice daily by subcutaneous injection. Ten normal rats were killed in order to obtain control data on thymus weights; 10 adrenalectomized rats were maintained for 7 days without treatment; 5 adrenalectomized rats were treated with 2 mg daily of 17-hydroxy-11-dehydro-corticosterone acetate; 5 adrenalectomized rats were treated with 2 mg of 11-desoxy-corticosterone; and 5 adrenalectomized rats were treated with 10.0 mg daily of 11-desoxy-corticosterone. Necropsy was performed on the 7th day. The data on body weights and thymus weights are summarized in Table I.

* I wish to express my appreciation to Dr. H. L. Mason of the Mayo Clinic who supplied the sample of 17-hydroxy-11-dehydro-corticosterone acetate; and to Dr. E. Oppenheimer of the Ciba Pharmaceutical Products, Inc., who supplied the 11-desoxy-corticosterone acetate.

TABLE I.
Body-Weights and Thymus Weights of Adrenalectomized Rats.

Exper. group	No. animals	Body wt		Thymus wt	
		Avg	Range	Avg	Range
Normals	10	180	179-181	447.0	358-576
Untreated	10	153.8	143-173	387.0	302-523
2.0 mg daily 17-hydroxy-11-dehydro-corticosterone	5	156.4	148-171	24.6	21-27
2.0 mg daily 11-desoxy-corticosterone acetate	5	200.4	198-203	341.0	285-395
10 mg daily 11-desoxy-corticosterone acetate	5	194.4	186-199	239.0	203-295

Although 2 mg daily of 17-hydroxy-11-dehydro-corticosterone acetate did not protect the adrenalectomized rat against a loss in body weight it did produce a marked regression of the thymus. A similar dose of 11-desoxy-corticosterone acetate permitted the adrenalectomized rat to gain in weight during the period of treatment but it did not produce a significant regression of the thymus. The administration of 10 mg daily of 11-desoxy-corticosterone acetate did produce a definite loss in thymus weight but the extent of atrophy was much less than that produced by the 2.0 mg daily dose of 17-hydroxy-11-dehydro-corticosterone acetate. Selye¹ has previously observed a regression of the thymus following the administration of 11-desoxy-corticosterone to rats.

Wells and Kendall² reported that the administration of 11-desoxy-corticosterone acetate to normal rats did not cause regression of the thymus, whereas positive effects were obtained by the administration of corticosterone and its acetate. These workers administered the 11-desoxy-corticosterone acetate in a different manner than was used in this experiment and they did not administer amounts as large as 10.0 mg daily. Ingle and Mason³ had previously noted regression in the weight of the thymus following the administration of 17-hydroxy-11-dehydro-corticosterone in solid form to the normal rat. The quantitative relationship of these compounds in respect to the effect upon the thymus is similar to that of their effect upon the capacity of the adrenalectomized rat to work^{4, 5} and differs from their relative life maintenance activity.

¹ Selye, H., *The Canadian Med. Assn. J.*, 1940, **42**, 113.

² Wells, B. B., and Kendall, E. C., *Proc. Staff Meet. Mayo Clinic*, 1940, **15**, 133.

³ Ingle, D. J., and Mason, H. L., *Proc. Soc. Exp. Biol. and Med.*, 1938, **39**, 154.

⁴ Ingle, D. J., *Endocrinology*, 1940, **26**, 472.

⁵ Ingle, D. J., *Endocrinology*, in press.

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Diabetogenic Effect of Some Cortin-Like Compounds.

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Studies by Long and his coworkers¹ demonstrated the diabetogenic effect of some of the steroid compounds occurring in the adrenal cortex. The effect of 11-desoxy-corticosterone acetate was found to be slight compared to that of corticosterone and 11-dehydro-corticosterone. Jensen and Grattan² have reported that 11-desoxy-corticosterone acetate is much less effective in preventing insulin convulsions in mice than are corticosterone and 11-dehydro-corticosterone.

In the following experiments partially depancreatized³ male rats having a body weight of approximately 280 g were used. The food intake of each animal was kept constant by administering the food

TABLE I.
Effect of Some Cortin-like Compounds on the Glycosuria of Partially Depancreatized Rats.

Substance	Daily dose, mg	Rat No.	Glycosuria g/day*		
			Before inj.	During inj.	Following inj.
11-desoxy-corticosterone acetate	1	7	0	0	0
	2	3	0	0	0
	2	3	0	0	0
	5	3	0	0	0
	5	5	1.75	1.75	1.11
	10	5	1.11	2.12	0.83
	5	8	1.00	3.80	1.78
	10	7	0.95	1.17	0.90
17-hydroxy-11-dehydro-corticosterone acetate	1	8	1.78	3.70	†
	2	2	2.63	4.20	†
	2	3	0	2.12	0
	2	11	0	4.7	†
	5	3	0	5.50	0
17-hydroxy-corticosterone	2	7	0.90	5.80	†

*The highest single value for daily excretion occurring prior to injection; the highest value for the response elicited by the injection; and the highest value following the injection period are given here.

†These experiments were terminated by the development of a severe ketonuria. Rats 2, 7, and 11 succumbed. Rat 8 was treated with insulin and recovered.

¹ Long, C. N. H., Katzin, B., and Fry, E., *Endocrinology*, 1940, **26**, 309.

² Jensen, H., and Grattan, J. F., *Am. J. Physiol.*, 1940, **128**, 270.

³ Shapiro, R., and Pincus, G., *Proc. Soc. Exp. Biol. and Med.*, 1936, **34**, 416.

by stomach tube in the manner described by Reinecke, Ball and Samuels.⁴ The test substances were dissolved in sesame oil and administered by subcutaneous injection twice daily. Each series of injections was continued for 4 days. The results are summarized in Table I.

The compound 17-hydroxy-11-dehydro-corticosterone appears to be more potent than 11-desoxy-corticosterone in its diabetogenic effect. In the one test of 17-hydroxy-corticosterone the increase in the glycosuria was very marked, indicating that this substance is also very active. Jensen and Grattan⁵ have observed that the anti-insulin effect of 17-hydroxy-11-dehydro-corticosterone and 17-hydroxy-corticosterone is greater than the anti-insulin effect of 11-desoxy-corticosterone.

The relative effects of the cortin-like compounds on carbohydrate metabolism parallel very closely their effects upon the capacity of adrenalectomized rats to work⁶ but differ from their relative maintenance activity.

The author is grateful to Miss Dorothy Quinn for technical assistance; Dr. H. L. Mason of the Mayo Clinic, who supplied the sample of 17-hydroxy-11-dehydro-corticosterone acetate and 17-hydroxy-corticosterone; and Dr. E. Schwenk of the Schering Corporation, Bloomfield, New Jersey, who supplied the 11-desoxy-corticosterone acetate.

⁴ Reinecke, R. M., Ball, H. A., and Samuels, L. T., PROC. SOC. EXP. BIOL. AND MED., 1939, **41**, 44.

⁵ Jensen, H., and Grattan, J. F., personal communication.

⁶ Ingle, D. J., *Endocrinology*, in press.

Alcoholate of Trimer of Hydroxypyruvic Aldehyde as Antidote in Mercuric Chloride Poisoning.

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A method for the purification of the alcoholate of the trimer of hydroxypyruvic aldehyde¹ and a study of the metabolism of this compound² have been reported.

The compound depolymerizes readily in aqueous solution, yielding 2 mols of hydroxypyruvic aldehyde and 1 mol of the alcoholate of the monomer and will therefore be referred to as hydroxypyruvic aldehyde. The aqueous solution reduces mercuric chloride rapidly in the cold, especially in the presence of disodium phosphate.

This investigation was undertaken to determine the capacity of hydroxypyruvic aldehyde and disodium phosphate to act as an antidote in experimental mercuric chloride poisoning.

Experimental. The experiments were performed on rabbits and cats. All animals were fasted during the 18 hours prior to experimental use, excepting 2 rabbits which were fasted for 48 hours. Mercuric chloride was administered by stomach tube in all of the experiments. Hydroxypyruvic aldehyde and disodium phosphate were given consecutively, whether administered orally or intravenously. Oral doses were followed by a wash of 1 cc of water.

1. *Rabbit Experiments.* In this series of experiments mercuric chloride was administered in 1% solution, hydroxypyruvic aldehyde in 5% solution and disodium phosphate in 5% solution.

Blood urea-nitrogen levels were determined by Karr's direct Nesslerization method.³ These are recorded in Table II.

The respective groups of rabbits were treated with the antidote orally and intravenously 15, 30, and 60 minutes after the administration of mercuric chloride. These dosages are recorded in Table I. In most cases oral treatment with the antidote was repeated.

2. *Cat Experiments.* In this series of experiments each cat received 5 mg of morphine sulfate per kg of body weight subcutaneously

¹ Evans, W. E., Jr., Carr, C. J., and Krantz, J. C., Jr., *J. A. C. S.*, 1938, **60**, 1628.

² Evans, W. E., Carr, C. J., and Krantz, J. C., Jr., *Proc. Soc. Exp. Biol. and Med.*, 1938, **39**, 573.

³ Karr, W. G., *J. Lab. Clin. Med.*, 1924, **9**, 3.

TABLE I.
Dosages of Substances Administered to Rabbits.

Substance	Dosage (mg/kg)	
	Oral	Intravenous
Mercuric Chloride	20	0
Hydroxypyruvic Aldehyde	500	125
Disodium Phosphate	250	65

one hour before the experimental poisoning in order to prevent vomiting. Mercuric chloride was administered in 2% solution and hydroxypyruvic aldehyde and disodium phosphate in 10% solution.

Groups of cats were treated with hydroxypyruvic aldehyde and disodium phosphate at intervals of 1, 5 and 15 minutes respectively, after the administration of a fatal dose of mercuric chloride. The results are shown in Table III.

Discussion. Autopsies performed on rabbits No. 10, No. 21, and No. 24 showed no gross pathology of the gastrointestinal tract or kidneys. On examination of rabbit No. 10 the liver was found to be highly parasitized and there were hemorrhagic patches on the lungs. Death of rabbits No. 23 and 25 on the eleventh day of the experiments may have been caused by unusually hot weather. This conclusion is supported by the blood-urea-nitrogen determinations made on the ninth day. These determinations indicated that there was slight or no renal impairment at this time.

Treatment of 11 rabbits with hydroxypyruvic aldehyde and disodium phosphate one hour after the administration of mercuric chloride prevented renal injury in 9 animals. Decreased chance of survival after prolonged fasting is indicated by the blood urea-nitrogen levels of the 2 animals which had been fasted for 48 hours prior to experimental poisoning. Oral treatment with the antidote again on the second day of the experiment may favor recovery.

Oral treatment of 11 cats with hydroxypyruvic aldehyde and disodium phosphate one minute after the administration of mercuric chloride prevented acute poisoning. Nine animals survived longer than 30 days. The other 2 cats died on the seventeenth and twenty-fourth days, respectively, of the experiment. The antidote did not protect cats effectively if 5 minutes had elapsed before treatment but did increase the average survival time. Hydroxypyruvic aldehyde and disodium phosphate had no antidotal action when administered 15 minutes after mercuric chloride had been given.

Conclusions. Hydroxypyruvic aldehyde in the presence of disodium phosphate acted, within the limits set forth in these experiments, as an effective antidote against mercuric chloride poisoning in rabbits and cats.

TABLE II.
Blood Urea-Nitrogen Determined on Rabbits Poisoned with Mercuric Chloride and Subsequently Treated with Hydroxypyruvic Aldehyde and Disodium Phosphate.

No. rabbits in group	Time bet. adm. of poison and antidote, min.	Rabbit No.	Survival period, days	Blood urea-nitrogen (mg%)									Remarks
				Control	2	3	4	5	6	7	8	9	
3		6	3	16.8	—	218	534*						Only phosphate adm. Oral antidote repeated 2nd and 3rd days
		7	2	23.2	—								
		8	3	15.9	—	276							
3	15	12	1	17.4	—								Only phosphate adm. Oral antidote repeated 2nd and 3rd days
		13	>30	7.6	—	14.3	17.6	—	21.0				
		14	4	5.2	—	172	237						
3	15	9	>30	21.8	—	19.0	14.8	—	18.5				Oral antidote repeated 2nd and 3rd days
		10	4	6.0	—	32.1	24.2						
		11	>30	7.7	—	18.6	12.3	—	14.6				
3	30	15	>30	19.2	35.9	12.3	—	15.3					Oral antidote repeated 2nd day
		16	14	22.6	37.5	57.0	—	61.6					
		17	>30	18.8	33.2	16.3	—	10.4					
11	60	18	5	24.1	53.0	—	>100	—	279				Oral antidote repeated 2nd day
		19	>30	20.3	26.2	—	15.6	—		14.2			
		20	>30	13.2	20.4	—	13.9	—		12.4			
		21	>20*	11.4	20.6	—	13.6	—		12.0			
		22	>30	18.9	20.2	—	14.8	—		19.4			
		23	11	19.7	35.2	23.5	—	—				24.8	
		24	12*	16.7	38.0	47.5	—	27.3				30.6	
		25	11	11.3	22.2	—	—	15.9				14.4	
		26	1	14.6									
		33	>30	19.2	—	—	123	—	73.0	—		66.6	
		34	>30	17.2	—	—	82	—	47.5	—		52.8	

> More than.

* Sacrificed for autopsy.

TABLE III.
Antidote After Oral Administration of Mercuric Chloride to Cats.

Cats in group No.	Dose of mercuric chloride, mg/kg	Time interval bet. adm. of poison and antidote, min	Antidote (mg per kg)				Survival period		Avg survival period in fatal cases, days
			Hydroxypyruvic aldehyde		Disodium phosphate		Cats No.	Days	
			Oral	I.V.	Oral	I.V.			
3	25		0	0	0	0	1	1	1.7
							2	2	
3	0		250	62	125	31	3	>30	
6	25	5	250	62	125	31	2	1	8.5
							1	3	
							1	4	
							1	14	
							1	28	
5	25	15	250	62	125	31	1	1	2.0
							3	2	
							1	3	
12	25	15	500	0	250	0	5	1	2.3
							4	4	
							3	>30	
4	25	5	500	0	250	0	2	2	7.0
							1	10	
							1	14	
11	25	1	500	0	250	0	1	17	20.5
							1	24	
							9	>30	

Each cat received 5 mg of morphine sulfate per kg body weight subcutaneously one hour before mercuric chloride was administered.

Alterations in the Blood Histamine in Shock.

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Within the last decade much experimental data has accumulated on the subject of shock, and the explanation of its mechanism has been greatly clarified. There is still considerable controversy, however, about the actual factors responsible for the production of this syndrome. A discussion of the prevalent theories cannot be undertaken here for reasons of space but excellent reviews are available. (Moon.¹)

In an effort to investigate the relationship of histamine to various clinical conditions, the total blood histamine of a series of normal individuals was determined, using the method of Barsoum and Gaddum² as modified by Code.³ Since the production of reactive hyperemia by prolonged stasis of the circulation has been demonstrated to increase the blood histamine (Barsoum and Smirk⁴), specimens were obtained observing precautions against stasis. In a series of 50 controls the average figure was found to be 0.04 γ /cc (as base) with variation of from 0.025 to 0.08 γ /cc. These figures agree in general with those of Haworth and MacDonald,⁵ although they found a variation from 0.018 γ /cc to 0.078 γ /cc. In repeated examinations of the blood of the same individual from time to time over a period of months, it has been found that the blood histamine remains at a fairly constant level. For example, in a patient whose first sample contained 0.07 γ /cc subsequent samples at intervals of 2 days contained 0.07, 0.065 and 0.07 γ /cc. Three more at weekly intervals and a later one taken 2 months after the initial examination all gave values of 0.07 γ /cc. In several of the patients, one with an initial blood histamine value of 0.05 γ /cc and another with 0.04 γ /cc similar results were obtained. In a series of 150 patients with various conditions, 19 cases presenting shock varying from a mild form to severe collapse as determined by clinical signs and blood

¹ Moon, V. H., *Ann. Int. Med.*, 1938, **12**, 205; *Shock and Related Capillary Phenomena*, Oxford Univ. Press, 1938.

² Barsoum, G. S., and Gaddum, J. H., *J. Phys.*, 1935, **85**, 1; *Clin. Sci.*, 1936, **2**, 357.

³ Code, C. F., *J. Phys.*, 1937, **89**, 257.

⁴ Barsoum, G. S., and Smirk, F. H., *Clin. Sci.*, 1935, **2**, 353.

⁵ Haworth, E., and MacDonald, A. D., *J. Hyg.*, 1937, **37**, 234.

studies including plasma specific gravity, hemoglobin and hematocrit estimations were studied. In 4 cases, a single determination only was obtained. Control specimens were taken either before operation or after recovery in the remaining 15. These have been divided into 3 groups. Table I gives the histamine values obtained on 8 patients who underwent surgical operation without manifesting any clinical or other signs of shock. One case of severe trauma in a child of 8 is also included.

It will be observed that little or no change in the blood histamine occurred in 5 of these patients. In 2, however, (No. 155 and 162) a moderate decrease occurred. Blood studies were performed simultaneously and in no instance was there any degree of hemoconcentration. One exception in this group is patient No. 96 in whom a single determination only was obtained several hours after operation and the value for blood histamine was 0.015 γ /cc, which is low as compared to the normal average. He did not have any symptoms of shock.

TABLE I.

Blood Histamine in Patients Undergoing Surgical Operation Without Developing Shock.

Case No.	Age	Sex	Operation	Time of specimen	Blood histamine γ /cc
70	30	F	D and C	Control	.05
				1 hr	.03
96	32	M	Nephrectomy	4 hr	.015
117	30	M	Gastric resection	Control	.06
				4 hr	.05
134	34	M	Gastric resection	Control	.065
				1 hr	.075
				2 hr	.05
				24 hr	.05
148	45	M	Gastric resection	Control	.035
				3 hr	.042
155	36	F	Thoracotomy	Control	.062
				3:30 hr	.035
				24 hr	.033
				7 days	.025
				14 days	.042
162	50	F	Cholecystectomy	Control	.06
				2 hr	.05
				24 hr	.04
				2 days	.06
				8 days	.08
93	9	M	Trauma to leg followed by amputation	1 hr after trauma	.04
				1 hr after amputation	.04

TABLE II.
Blood Histamine in Patients Developing Shock Following Surgical Interference.

Case No.	Sex	Age	Operation	Time specimen taken	Signs of shock	Blood histamine γ /cc
20	M	25	Gastric resection	4 hr P.O. 3 days P.O.	+++ Recovered	.01 .05
26	M	21	Bilateral hydronephrosis	6 days P.O.	+++	.02
95	M	42	Gastric resection	12 hr P.O. 6 days P.O.	+++ Recovered	.01 .06
102	M	65	1st stage prostatectomy	5 hr P.O. 48 hr P.O. 7 days P.O.	+++ Recovered	.015 .08 .08
130	M	45	Abdomino-perineal resection	Control 1½ hr P.O. 24 " P.O.	++ Condition good	.035 .01 .04
141	M	48	Acute intestinal obstruction, patient operated on	24 hr 2 " P.O. 24 " P.O. 48 " P.O. 72 " P.O.	+++ + ++ +++ died	.01 .06 .025 .01
161	F		Gastroduodenostomy	Control 3 hr P.O. 5 " P.O. 24 " 48 " 8 days	— ++ ++ — — —	.07 .035 .03 .03 .038 .07
116	M	31	Pyloroplasty	Control 24 hr P.O. 48 " 7 days 14 "	— — ++ ++ ++ —	.08 .08 .035 .04 .02 .04

TABLE III.
Cases of Traumatic Shock.

Case No.	Sex	Age	Trauma	Time specimen taken	Signs of shock	Blood histamine γ /cc
4	M	28	Industrial accident, hit by falling bricks	4 hrs after	+++	.002
118	M	50	Severe injury to right arm, with hemorrhage Operation amputation of arm	1 hr after trauma 4 hr P.O. 18 " P.O. 20 "	+++ ++ ++ died	.11 .04 .02
131	F		Head injury with hemorrhage		+++	.015

Eight patients who exhibited signs of shock following operation are presented in Table II. Six of these showed marked changes in the blood histamine, and this was also accompanied by evidences of hemoconcentration as determined by simultaneous blood studies. It will be observed that as a general rule, the blood histamine is low or decreasing within 2 to 3 hours following the operation and that there is a return to normal or even high levels after varying intervals of time.

In Table III are presented 3 patients admitted following severe trauma. Single determinations only were available on 2 of these, No. 4 and 131, and it will be noted that the blood histamine level is decreased. Case 118 was admitted to the hospital within 45 minutes after having sustained a severe traumatic injury complicated by hemorrhage. A specimen was obtained before any therapy was administered and the blood histamine was found to be 0.11 γ /cc. This is higher than the normal value. Following amputation of the right arm, and administration of cortin intravenously along with a transfusion, the blood histamine was again determined and found to be 0.04 γ /cc. On the following day, a third specimen was found to be 0.02 γ /cc at 10:30 A.M. and at 12:30 P.M. the patient died. It should be noted that in all other cases, the first examination of the blood was done only several hours after the trauma.

A single determination only was obtained in 6 cases in whom death occurred within 3 to 48 hours. These are presented in Table IV and it will be noted that the blood histamine is markedly decreased in all as compared to normal values.

The histamine theory of shock has been rejected by many different

TABLE IV.
Cases in Agonal States.

Case No.	Sex	Age	Diagnosis	Time of death after operation or onset of symptoms	Time before death specimen taken	Histamine γ /cc
86	F	45	Severe burns	7 days	24 hr	.001 \pm
111	F	33	Dehydration colitis		24 "	.015
119	M	40	Mesenteric thrombosis	36 hr	12 "	.015
123	F	35	Extreme cachexia	6 mo	48 "	.001
135	M	60	Ca of stomach		48 "	.01
167	F	30	Pneumonia and peritonitis following appendectomy	7 days P.O.	3 "	.005

workers mainly because of the difficulty of demonstrating the presence of an active depressor substance in the blood of experimental animals or in that of man during shock (Schneider,⁶ O'Shaughnessy and Slome,⁷ Dragstedt and Mead⁸). On the other hand, histamine is looked upon as a factor responsible for the production of symptoms of anaphylactic shock in the guinea pig (Bartosch, Feldberg and Nagel,⁹ Code¹⁰), and in the dog (Dragstedt and Mead,¹¹ Code¹⁰). In both of these species, anaphylactic shock is accompanied by an increase of the blood histamine. Furthermore, there is little difference between the symptoms of anaphylactic shock and histamine shock in either of these species or in the rabbit. Yet, in the rabbit, the onset of anaphylactic shock is accompanied by a marked decrease in the blood histamine (Rose and Weil¹²). This has also been shown to occur in the horse and calf (Code and Hester¹³). It may be of interest to note that according to Moon¹ there is a fundamental similarity in the pathology of anaphylaxis and shock, namely changes in capillary function and endothelium.

Previous reports on the blood histamine in cases of shock due to extensive burns have indicated that there is first an increase of blood histamine followed by a slow return to normal (Barsoum and Gaddum,² Code and MacDonald¹⁴). According to Barsoum and Gaddum there was no relation between this rise and secondary shock. Only one case with severe burns has been available in the present study, in this a simultaneous study of the blood histamine, hemoconcentration and blood electrolytes was made. (Case No. 86). The blood showed marked hemoconcentration, and no histamine was found. This specimen was obtained on the sixth day after the trauma and the patient died 48 hours later.

In view of the above results, there appears to be a mechanism whereby the blood histamine is diminished in conditions where the accepted criteria of shock exist, and in certain other cases shortly before death. The nature of this mechanism is not yet clear, but may possibly be due to a transfer of the histamine of the blood to the

⁶ Schneider, H., *Deutsche Z. f. Chir.*, 1930, **229**, 343.

⁷ O'Shaughnessy, H. L., and Slome, D., *Brit. J. Surg.*, 1935, **22**, 589.

⁸ Dragstedt, C. A., and Mead, F. B., *J. Am. Med. Assn.*, 1937, **108**, 95.

⁹ Bartosch, R., Feldberg, W., and Nagel, E., *Arch. f. d. ges. Physiol.*, 1932, **230**, 129.

¹⁰ Code, C. F., *Am. J. Physiol.*, 1939, **127**, 78.

¹¹ Dragstedt, C. A., and Mead, F. B., *J. Pharm. and Exp. Ther.*, 1936, **57**, 419.

¹² Rose, B., and Weil, P., *Proc. Soc. Exp. Biol. and Med.*, 1939, **42**, 494.

¹³ Code, C. F., and Hester, H. R., *Am. J. Physiol.*, 1939, **127**, 71.

¹⁴ Code, C. F., and MacDonald, A. D., *Lancet*, 1937, **233**, 730.

damaged area or to certain of the abdominal viscera. This may possibly account for the failure to demonstrate an increase in the histamine content of the blood when shock is well established, although some of the cases indicate that there may be an early increase. It may be that there is first a liberation of histamine which is rapidly followed by a decrease of the blood histamine below the normal value. This could be accounted for if one assumed that the histamine gathers in the tissues, or traumatized area, as it does in areas of inflammation (Tarras-Wahlberg,¹⁵ Rocha e Silva and Bier¹⁶). Furthermore, in dogs it has been shown that following burns there is first an increase of the blood histamine, followed by a disappearance in a matter of 2-3 days, accompanied by an increase in the histamine content of the spleen, liver and pancreas (Kisima¹⁷). There is a marked similarity between adrenal insufficiency and shock and it has been demonstrated that the histamine content of the gastro-intestinal tract increases to 250% over the normal in adrenalectomized rats, although there is little change in the blood histamine of these animals.

It should be pointed out that although the blood histamine is markedly diminished in patients where shock is established, such a diminution may occur in certain other conditions in cases of allergy. In these latter states, however, one does not observe as marked a variation in the histamine content of the blood. This will be discussed in a subsequent communication.

Conclusions. In a series of patients in varying types of shock manifested by clinical signs, hemoconcentration, and lowering of the B.P. the total blood histamine has been determined. The results indicate that in the cases studied a marked diminution of the histamine content of the blood occurs when shock is established as compared with control values and those after recovery. There appears to be some correlation between the severity of the shock and the degree of blood histamine decrease. Blood histamine has also been found to be low in agonal states. The significance of these findings is discussed.

¹⁵ Tarras-Wahlberg, B., *Klin. Wschr.*, 1937, **16**, 958.

¹⁶ Rocha e Silva, M., and Bier, O., *Arq. do Inst. Biology*, 1938, **9**, 123.

¹⁷ Kisima, H., *Fukuoka Acta. Medica*, 1938, **31**, 49.

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Extracts of Anterior Pituitary Growth Hormone.

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The solvent action of phosphate buffer, urea and sodium hydroxide solutions in extracting the growth principle of the anterior pituitary gland was investigated with the aid of a comparatively simple bioassay method.¹ The potency of each extract was determined and expressed as mg per cc of a standard anterior lobe powder. The percent yield was calculated by dividing the potency by the number of mg of powder used per cc in making the extract.*

To facilitate comparison, all extracts were prepared in as uniform a manner as possible from the same desiccated, powdered anterior lobe substance† which also served as the standard for all assays. For example, phosphate buffer extract No. 1 (Table I) was prepared by extracting 25 g of powder with 200 cc of a solution (pH 8) containing 5.616 g of anhydrous Na_2HPO_4 and 0.302 g of anhydrous KH_2PO_4 per liter. After 3 hours of continuous stirring at 10°C, the insoluble residue was separated by centrifuging and successively extracted with three 125 cc volumes of buffer solution. The combined extract, which had a pH of 7.5, was filtered through cellulose and asbestos pads, sterilized by filtration through a Seitz apparatus and stored at 10°C in sterile rubber-capped bottles. After

¹ Light, A. E., deBeer, E. J., and Cook, C. A., in press.

* Inspection indicates that the dose-response curve (Fig. 1) for the extracts resembles that for the powder suspension. An experiment sufficiently elaborate to establish the linearity of the relationship between log dose and response will be reserved for a selected extract since it is obviously impractical to do this for each extract. However, at least 2 and often 4 graded doses of each extract were given (intraperitoneally) thus establishing the slope for each extract curve. The standard was employed in each assay. In the following example, the standard contains 5 mg of powder per cc.

Standard		Extract 8D	
Dose cc/100 g rat	Response % gain in wt	Dose cc/100 g rat	Response % gain in wt
0.05	1.3	0.01	2.0
0.10	3.0	0.02	3.4

Therefore, 1 cc of extract containing about 10 mg of protein is equivalent to 6.4 cc or 32 mg of standard powder.

† Burroughs Wellcome & Co. (The Wellcome Foundation, Ltd.), London.

TABLE I.
Extracts of Anterior Pituitary Growth Hormone.

No.	Preparation	Mean potency* mg/cc	Mean Yield %	Limits of error as % of mean		Protein Trichloro- acetic acid %	Total solids %	Ash %	Nitrogen (Kjeldahl) %
				Lower %	Upper %				
	Desiccated anterior lobe (standard)						88.0	5.0	11.5
1	Phosphate buffer	50.0	100	58	173	1.18	1.57	0.52	0.13
2	Phosphate buffer	39.2	78	51	196	1.43	1.80	0.70	0.14
3	NaOH	25.8	52	61	165	1.47	1.42	0.40	0.14
4	1% Urea	23.4	47	69	145	1.00			
5	5% "	20.0	40	58	172	1.00			
6	10% "	50.0	100	59	168	0.99			
6D	10% "	36.8	74	53	189	0.99			
7	20% "	30.5	61	64	156	1.15			
8	10% "	32.0	64	69	145	1.21	1.63	0.54	0.32†
8D	10% "	35.4	71	66	151	1.63			
9	10% "	33.8	68	75	134	1.12			
9D	10% "	33.8	77	58	173	1.00			
10†	10% "	19.2	77	58	173				0.31†
11	1% Guanidine	20.3	41	56	177				

* Mean potency determined in terms of mg of standard powder per cc of extract.

† 0.28% urea still present.

‡ Double volume.

D Dialyzed to remove urea.

several weeks a precipitate appeared in this extract. The high yield indicates that most of the active material was extracted. About 1% of the extract was organic matter, probably protein. This figure was approximated by 3 different methods, *i.e.*, by trichloroacetic acid precipitation, by calculating the total N as protein and by subtracting the ash, which was largely due to buffer salts, from the total solids.

In contrast to the buffer extracts, the gelatinous nature of the extracts prepared with dilute aqueous NaOH solutions made them very difficult to clarify by filtration. Furthermore, a considerable precipitate formed after one week of storage. In preparing this type of extract, NaOH was added at intervals in order to maintain the pH at 7.5. These extracts were not as uniform in potency as those obtained with phosphate buffers.

Urea, in high concentrations has been shown to bring about remarkable changes in the chemical and physical properties of certain proteins.^{2, 3} Accordingly, a study was made of the properties of phosphate buffer extracts containing 1%, 5%, 10%, and 20% urea. (Table I.) These preparations were of about the same potency as the phosphate buffer extracts. The addition of urea in concentrations as low as 1% or 5% retarded precipitation for several months, particularly if the urea were added just before filtration through a Seitz apparatus. When the concentration of urea was 10% or 20%,

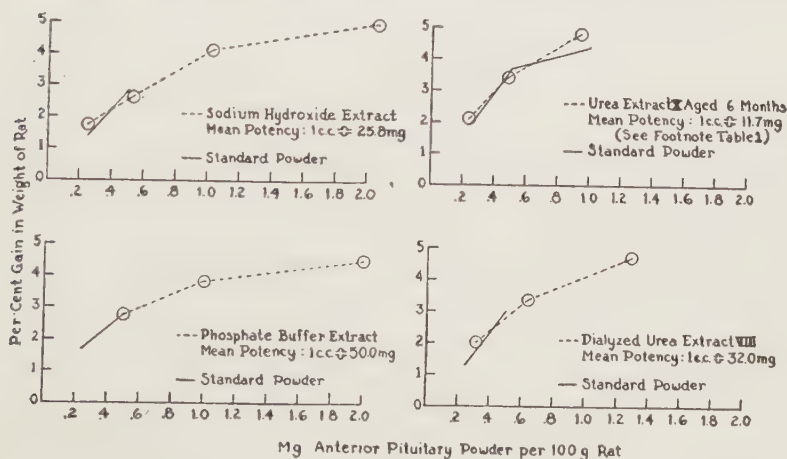


FIG. 1.

Dose response curves for anterior pituitary extracts in terms of standard powder and compared with standard powder curves.

² Steinhardt, J., *J. Biol. Chem.*, 1938, **123**, 543.

³ Greenstein, J. P., *Ibid.*, 1939, **128**, 233.

the extracts were still clear at the end of 6 months. When extracts 6 and 9 were dialyzed through cellophane membranes about 95% of the urea was removed and precipitates appeared in the corresponding preparations, 6D and 9D, in about 3 weeks.

As extracts 9 and 10 indicate, it appeared unnecessary to increase either pH or volume in order to improve the efficiency of the extraction. The 1% guanidine extract yielded an amount of hormone similar to that of the 1% urea.

Summary. Phosphate buffer extracts of growth hormone were highly active when assayed in terms of anterior pituitary powder. This method of extraction permitted a careful control of pH and gave high yields of hormone. These extracts had less tendency to form precipitates than those prepared with sodium hydroxide. The addition of urea retarded such precipitation.

11398

Biological Assay of Anterior Pituitary Growth Hormone.

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(Introduced by L. Reiner.)

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Normal animals have been employed in the assay of growth hormone by Evans *et al.*,¹ Van Dyke and coworkers² and Lee.³ Inherent limitations of such an assay method have emphasized the importance of statistical treatment of the data. Bülbring,⁴ working with hypophysectomized rats, has utilized the rapidly rising portion of a dose-response curve and has reported results which indicated relatively low limits of error.

In order to avoid the complex metabolic derangements associated with an extirpation of the entire pituitary gland, an assay procedure was developed in which groups of normal rats were used to determine the increased body weight resulting from administration of

¹ Evans, H. M., Uyei, N., Bartz, Q. R., and Simpson, M. E., *Endocrinology*, 1938, **22**, 483.

² Chou, C., Chang, C., Chen, G., and Van Dyke, H. B., *Ibid.*, 322.

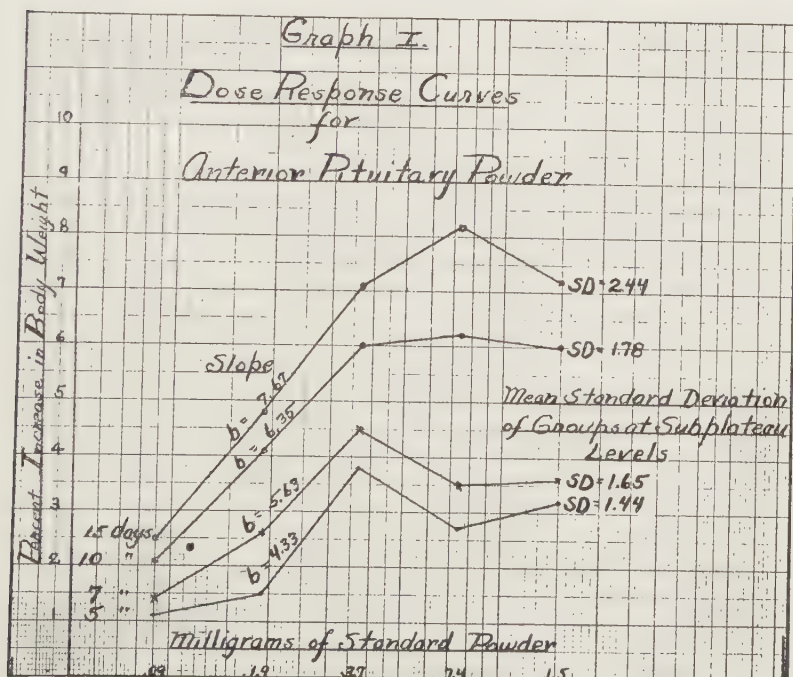
³ The Pituitary Gland, Proc. Assn. for Research in Nervous and Mental Disease, **17**, Williams & Wilkins, Baltimore, 1938, 216.

⁴ Bülbring, E., *Quart. J. Pharm. and Pharmacol.*, 1938, **11**, 26.

growth hormone preparations. Groups of 15 normal, plateaued, female rats, approximately 5 months of age, were selected with respect to strain (Yale), weight (240-300 g) and diet (Fox Chow). Three preliminary weighings during a 5-day period were necessary to establish the fact that a plateau level in the normal growth curve, essential for the assay method, had been reached. To accustom the animals to the standardized routine treatment, injections were also made at these times with saline, phosphate buffer (pH 8) or 10% urea in 0.04 molar buffer solution.

Well-defined dose-response curves were obtained when daily, graded doses of a saline suspension of desiccated anterior lobe substance* were injected intraperitoneally (Graph I). A sufficient quantity of this material was also available to serve as a standard for subsequent assays. Statistical analysis of a curve based on results obtained with 165 rats revealed that on the steeply ascending portion of the curve, below the plateau level, the per cent increase in body weight was a linear function of the logarithm of the dose.

In routine assays, 2 groups were injected with sub-plateau doses



* Burroughs Wellcome & Co. (The Wellcome Foundation, Ltd.), London.

of the standard powder* and two with the experimental extract. For each preparation the ratio of the greater dose to the smaller should be at least 2 to 1. The standard powder was suspended in a saline, buffer or urea solution and the assay data from 5 daily injections were used to calculate the potency of experimental preparations in terms of milligrams of the standard powder. Final group weights were recorded 120 hours after the initial injections of either the standard powder or the extracts. The groups were also weighed daily at the time of injection for evidence of any irregularity in body weight. Control groups injected with saline, 10% buffer solution or various doses of an inactive pituitary extract showed no significant changes in body weight.

Gaddum⁵ has shown that the statistical evaluation of bioassays involving a linear relationship and the use of a standard substance can be greatly simplified by following the plan given below. Using 4 groups, each containing an equal number of animals, let X_{11} , X_{12} be 2 doses of the standard preparation and X_{21} , X_{22} the doses of the test preparation, and the corresponding mean responses of each group be Y_{11} , Y_{12} , Y_{21} and Y_{22} . The responses were expressed as per cent gain in body weight. Let d , the log of the ratio of the greater to the smaller dose, *i. e.*, $\frac{X_{22}}{X_{21}}$, be the same for each preparation. The following equation will then express the potency of the test preparation in terms of cc of the standard solution.

$$1 \text{ cc of test solution} = \frac{X_{11}}{X_{21}} \text{ antilog} \left(\frac{Y_{21} + Y_{22}}{2b} - \frac{Y_{11} + Y_{12}}{2b} \right)$$

The term b , or slope, serves to convert each average response, *i. e.*, $\frac{Y_{21} + Y_{22}}{2}$ and $\frac{Y_{11} + Y_{12}}{2}$, into the logarithms of the corresponding average doses. Since the resulting terms are logarithmic, the difference between the two values represents the antilog of the ratio of the two average doses. The average slope, b , is given by the expression,

$$b = \frac{1}{2} \left(\frac{Y_{22} - Y_{21}}{d} + \frac{Y_{12} - Y_{11}}{d} \right).$$

The standard error of the assay, expressed logarithmically, is $\text{S.E.} = \frac{\text{S.D.}}{b} \frac{1}{\sqrt{n}}$, where S.D. is the standard deviation, b is the slope and n is the number of animals in a single group. When restricted to the sub-plateau levels, the standard deviation was independent of the size of the dose. The limits of error within which the calculated

⁵ Gaddum, J. H., Med. Res. Council, Special Report Series No. 183, 1933, 31.

potency should fall 21 out of 22 times, can be obtained by dividing the potency by the antilog of 2 S.E. for the lower limit and multiplying the potency by the antilog of 2 S.E. for the upper limit.

As shown in Graph I, a longer period of treatment results in an increased slope of the sub-plateau portion of the curve. This would tend to narrow the limits of error but it is partly offset by an increase in the standard deviation. The limits of error when calculated as described above were 67-149, 70-142, 71-140 and 68-147% of the mean potency for the 5-, 7-, 10- and 15-day periods of treatment, respectively. These limits of error have been confirmed by additional assays with normal rats. The average of 31 standard deviations was 1.6 for the 5-day assay. In view of these results, the 5-day period was used for routine assays. Bülbring's data from hypophysectomized rats gave limits of 49-206% for the 7-day period when calculated as above. These wider limits are largely due to the fact that Bülbring used only 5 animals per group instead of 15 as used in the present study, since the ratio $\frac{\text{S.D.}}{b}$ is approximately the same for both sets of data. Bülbring's ratio is $\frac{4.95}{14}$ or 0.354. The corresponding figures from Graph I are $\frac{1.65}{5.63}$ or 0.293. The actual values for standard deviation and slope are not comparable since Bülbring expresses the response in grams, while in Graph I response is given in per cent.

In comparing 2 preparations assayed against the same standard, but at different times, the standard error, by the formula⁶ for the standard error of the difference of 2 means is

$$\text{S.E.}(M_1 - M_2) = \sqrt{(\text{S.E.}_{M_1})^2 + (\text{S.E.}_{M_2})^2}$$

is the S.E._{M_1} is the standard error of one assay and S.E._{M_2} where standard error of the other assay. Since these values are approximately equal, the standard error is the $\sqrt{2}$ or 1.41 times the standard error of a single assay, which for a 5-day period gives limits of about 57-174%. This means that for 2 preparations, each assayed against the same standard powder, one must be nearly twice as potent as the other in order to be considered significantly different. Evidence is being accumulated as to the stability of the hormone in the anterior pituitary powders and in experimental extracts.

⁶ Coward, K. H., *Biological Standardisation of the Vitamins*, Balliere, Tindall and Cox, London, 1938, 166.

Summary. A selected anterior lobe powder from ox pituitary was used successfully as a standard of reference for one year in an assay procedure employing normal rats. By restricting the biological comparison to the steeper portion of the dose-response curves it was possible to obtain relatively low limits of error.

11399 P

Application in Man of Method for Continuous Reciprocal Transfusion of Blood.*†

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Purified heparin, by prolonging the coagulation time of blood, has reduced the technical difficulties and made feasible the continuous reciprocal transfusion of blood in man. Thalhimer, Solandt and Best,¹ using a uremic and a normal dog and employing purified heparin as an anticoagulant, conducted a reciprocal transfusion for 27 hours, reducing the blood urea from high levels to normal without injury to the normal dog. Prinzmetal² carried out exchange transfusions in the investigation of arterial hypertension in patients with inoperable cancer. We have been unable however, to find any instance in which the work of Thalhimer *et al.* was applied in man.

Heparin (10,000 Toronto units in 1,000 cc normal saline) was given intravenously at approximately the rate of 40 drops per minute to the normal donor and to the patient for 20 minutes before and throughout the transfusion. An additional 2,000 units were given intravenously to each individual as the transfusion was started, and repeated 30 minutes later. This maintained the blood coagulation time between 20 and 30 minutes. The median basilic veins of the

* The authors are indebted to Dr. William A. Wolff, Chemist to the Pennsylvania Hospital, for valuable suggestions on the chemical aspects of this problem.

† Since this paper was submitted for publication a transfusion was carried out in which 26,770 cc were exchanged in approximately 5 hours. The total nitrogen excretion in the urine increased from 548 mg per hour before to 851 mg per hour during the transfusion.

¹ Thalhimer, W., Soldant, D. G., and Best, C. H., *Lancet*, 1938, **2**, 554.

² Prinzmetal, M., Friedman, B., and Rosenthal, N., *Proc. Soc. Exp. Biol. and Med.*, 1936, **34**, 545.

two, a uremic patient and a normal donor, were then connected by a suitable rubber tubing filled with heparin in saline. The tubes passed through a roller pump which milked forward the same quantity of blood in opposite directions. A 400 mm manometer was placed between the pump and the recipient (1B and 2C), thus providing a sensitive indicator of any obstruction in the needle and at the same time a safeguard against undue increasing pressure in the receiving vein (Fig. 1).

Sufficient bicarbonate of soda was given to the donor and the recipient to make the urine alkaline as a preparatory measure. Trials of the apparatus in artificial models gave good results. Two patients with chronic uremia, one as a result of a polycystic disease of the kidneys and the other from chronic glomerular nephritis, have been given continuous reciprocal transfusions from normal donors. In the first patient an exchange of 2,520 cc of blood (including saline and heparin) was made over a period of 2 hours and 50 minutes. On the second patient 7,020 cc of blood, not including saline and heparin, were cross transfused in 47 minutes; during the greater portion of the time 82 cc of blood were delivered in each direction per

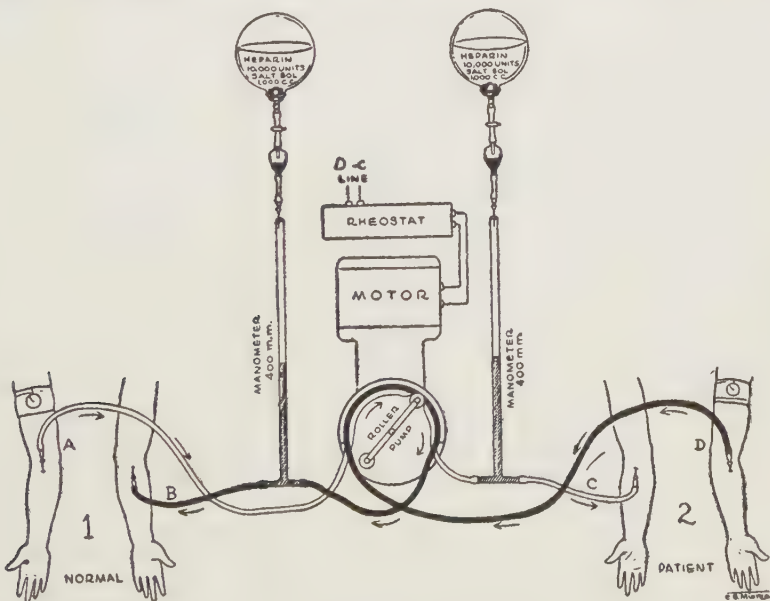


FIG. 1.

Diagram of the apparatus. 1A—delivering vein, 1B—receiving vein of the normal individual. 2D—delivering vein, 2C—receiving vein of the uremic patient. The 2 tubes rest exactly superimposed in a semi-circle around the periphery of the roller pump.

minute. In neither study were there any untoward reactions, except a slight elevation of temperature in one of the normal donors, attributed to a large hematoma having occurred when the needle escaped from the receiving vein.

Clinically both patients seemed improved. In Table I are listed the average hourly excretion of nitrogen in the urine of the two patients and the normal persons, as an example of the metabolic changes induced by the reciprocal transfusions. Further observations by this method, now under way, may help to elucidate obscure points in various metabolic disorders and perhaps make it possible to afford some relief during acute, though transient, incapacitations of the urinary apparatus.

TABLE I.
Total Nitrogen Excretion (mg per hr) in the Urine of the 2 Uremic Patients and the 2 Normal Donors Before, During and After the Reciprocal Transfusion.

Period	Nitrogen Excretion (mg per hr)							
	Uremic Patient (M.B.)				Normal Donor (A.P.)			
	Total N	Urea N	Uric Acid	Creatinine	Total N	Urea N	Uric Acid	Creatinine
12 hrs prior to transfusion	230	190	5.6	13.5	306	232	3.9	21.4
4 $\frac{5}{6}$ hr including transfusion period	322	229	5.6	16.8	471	428	15.0	32.5
Subsequent 6 hr	301	246	4.6	18.8	342	316	8.0	30.0
6-18 hr after transfusion	253	227	—	15.1	399	356	—	31.4
	Uremic Patient (S.C.)				Normal Donor (E.C.)			
13 hr 55 min prior to transfusion	319	262	2.6	13.5	398	340	1.0	16.5
3 $\frac{3}{4}$ hr including transfusion period	478	339	4.3	16.3	486	412	6.3	—
6 hr following this period	191	118	1.3	10.0	302	173	6.3	30.0
Subsequent 6-19 $\frac{1}{2}$ hr period	262	228	1.9	—	378	239	4.6	20.0

The Rôle of Boron in the Diet of the Rat.*

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Boron is of practically universal occurrence in the plant kingdom. Hence it is apt to be present in almost all classes of foodstuffs, and therefore occurrence of this element might be equally widespread in the animal kingdom and perhaps might play an equally important rôle in the physiology of the animal organism. At present little is known regarding the action or even the distribution of boron in animals. There are but few reports on the distribution of this element in animal tissues.¹⁻⁴ These few briefly-reported experiments indicate the necessity for further knowledge of the rôle of boron in the animal organism. As far as was known at the time this investigation was started, no data were on record as to whether boron is a dietary essential for animals. It seemed advisable, therefore, to determine whether boron is required for the growth and development of the rat or whether it is present in the animal body as an accidental constituent ingested with all foods.

Experimental. Experience with the study of other trace elements has shown that to do so effectively, a diet extremely deficient in the element must be used. The natural foods commonly used in purified rations were too abundant in boron. Cow's milk, although lower in this element, is not as low as is desirable and furthermore, a dry ration is preferable for such studies. Hence an attempt was made to produce such a diet depleted of its boron content. Naftel's⁵ micro method, using a photoelectric colorimeter, was employed for the determination of boron in the various foods studied. The ashing procedure was somewhat modified by using the overhead heater which has proven advantageous in the matter of time saved and in the prevention of loss of boron due to excessive smoking and swelling

* This work was aided by a grant from the Rockefeller Foundation.

¹ Bertrand, G., and Agulhon, H., *Compt. rend.*, 1912, **155**, 248; 1913, **156**, 732, 2027.

² Wright, N. C., and Papisch, J., *Science*, 1929, **69**, 78.

³ Blumberg, H., and Rask, O. S., *J. Nutr.*, 1933, **6**, 285.

⁴ Drea, W. F., *J. Nutr.*, 1934, **8**, 229; **10**, 351; **16**, 325.

⁵ Naftel, J. A., *Ind. and Eng. Chem. Anal. Ed.*, 1939, **11**, 407.

during the preliminary heating of the material. The overhead heater was also used instead of the water bath described in the original method, for it was found that the temperature could be much more easily controlled by this means. First, a survey was made of purified natural foodstuffs used in the diet of the rat. The foods containing the smallest amounts of boron were selected and the removal of their boron was attempted. Strong acid and methyl alcohol were used for this purpose in an attempt to remove the boron in the form of the methyl borate. Investigation of the extraction of boron with methyl alcohol showed first that quantitative recovery of boron used by this means was seldom obtained. By increasing the number of extractions or decreasing the amount of food to be extracted the results were not improved. Furthermore, this procedure was found unsatisfactory, first because complete elimination of the element appeared impossible, and secondly, in the cases where the boron was largely separated from the food, particularly in the case of protein, the food was so altered in its nutritive properties that it was not satisfactory for nutritional studies. It was evident at this stage, therefore, that the best solution to this problem was the preparation of an adequate diet composed of foodstuffs as low in boron as could possibly be found without attempting the removal of the element.

At about this time the report of Hove and associates⁶ on their studies of boron in animal nutrition appeared. Observations were then available in this laboratory, using a diet in connection with an experiment conducted for other purposes, but the boron content of which was practically the same as that of the Wisconsin experimenters. The various components of this diet along with others used in our laboratory had been tested for boron. The composition of this ration was considerably different from that used by Hove and his co-workers.⁶ Its total boron content was 163 μg per kg as compared with 155 μg of the diet described by the above mentioned investigators.⁶ It was thought, therefore, that it might be of interest to record our findings as further evidence of the results already reported,⁶ particularly since the animals fed the boron-low diet in this laboratory have been observed for a longer period of time and in somewhat greater detail.

The lactalbumin was prepared by the Harris Laboratories from fresh centrifuged milk. It is a product of high chemical purity, its ash content being but 0.72%. It was found to be extremely low in boron. Wheat gluten and gelatin contained about the same concentration

⁶ Hove, E., Elvehjem, C. A., and Hart, E. B., *Am. J. Physiol.*, 1939, **127**, 689.

Composition of Diet		Composition of Salt Mixture No. 22	
Lactalbumin	10.0	CaCO ₃	1.08
Wheat gluten	4.0	CaHPO ₄ · 2H ₂ O	.72
Gelatin	4.0	KCl	.85
Salt mixture No. 22	5.7	MgSO ₄	.50
Purified sweet butter fat	8.0	NaH ₂ PO ₄ · H ₂ O	1.14
Dextrose	to 100	NaCl	1.24
Viosterol 15 drops per kilo		NaI	.00015
Thiamin hydrochloride — 20 µg		Na ₂ SiO ₃ · 9H ₂ O	.035
per rat per day		FeSO ₄ (NH ₄) ₂ SO ₄ · 6H ₂ O	.07
Liver concentrate ≡ 3.5 g fresh liver		CuSO ₄ · 5H ₂ O	.025
per rat per day		MnSO ₄ · 4H ₂ O	.005
Vitamin E concentrate 3 mg per		ZnCl ₂	.01
rat per day			—
			5.7 g
			in 100 g diet

of boron as this highly purified lactalbumin; therefore these 3 were selected as the sources of protein for this diet. Dextrose was the carbohydrate found to be lowest in boron. Sweet butter fat remelted and filtered served as the source of fat and vitamin A. Viosterol, a liver concentrate† especially prepared by the Lederle Laboratories and vitamin E concentrate prepared by the Mackenzie method⁷ did not, in the amounts analyzed, show the presence of boron. The salt mixture contributed the greatest amount of boron of any of the ingredients of this diet.

Litter mates weighing 35-40 g were used in this experiment. These animals were housed in galvanized wire cages in the regular animal room of this laboratory. Monel metal or porcelain feeding cups and soft glass‡ drinking tubes were used.

Growth and reproduction on this diet were studied.

Extensive histological studies were also made on the tissues of these animals.

Results. The growth in body weight was observed and the food consumption and water intake were recorded; the average daily intake for the experimental period of 34 weeks being 9.6 g of diet per male and 7.8 g per female. The average daily boron intake was 1.56 µg per male and 1.27 µg per female. The general appearance of these animals was good and the rate of growth was normal. The growth of rats on this ration is shown in Table I. It is of interest to note that during the first 6 weeks these animals averaged a daily food intake of 5.2 g containing 0.85 µg boron. This compares well with the observations of the Wisconsin experimenters.

† I wish to thank the Lederle Laboratories, Inc., for supplying this material.

⁷ Mackenzie, C. G., Mackenzie, J. B., and McCollum, E. V., *U. S. Public Health Rep.*, 1939, **53**, 1779.

‡ Common soft glass is reported to be free from boron.⁸

⁸ Berger, K. C., and Truog, E., *Ind. and Eng. Chem., Anal. Ed.*, 1939, **11**, 540.

TABLE I.
Growth of Rats on Boron-low Diet.

	No. of rats	Avg wt in g per day	Avg daily food consumption in g	No. weeks avg
Males	12	3.4	9.6	34
Females	11	2.2	7.8	34

The females on this synthetic diet were allowed to carry through 3 litters each, averaging 7 young per litter which were born living, and normal in appearance and weight and which they successfully raised. Further matings were not carried out.

Detailed pathological studies of the tissues of the rats at the end of 34 weeks' experimental period showed that they were normal in every respect.

Since the diet has been used primarily in another investigation, the effect of supplementing it with boron in various concentrations has not been observed and, hence, no comparison can be made between the animals fed the diet described and rats receiving boron in greater concentrations.

The data presented here confirm the findings of Hove, Elvehjem and Hart⁶ that if boron is actually essential for growth and development of the rat, it must be in extremely small amounts since an average of 1.27-1.56 μg per rat daily for a period of 34 weeks apparently satisfied its requirement for normal function. It is possible that a deficiency of this element would become apparent in the second or later generations when the boron store might become markedly depleted.

Summary. A synthetic diet is described which has a boron content of 163 μg per kg. This diet supports good growth and development in the rat. Food consumption compares well with that of animals on good purified diets used in this laboratory. The reproductive processes are normal. The young born to mothers on this boron-low diet appear normal and are successfully raised. Pathological studies showed the tissues of these rats to be normal. No evidence is obtained under the experimental conditions that boron is essential in the nutrition of the rat.

Urinary Excretion of Ascorbic Acid by the Rat as Influenced by Ingestion of Certain Carbohydrates.*

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It is now recognized that the rat is not only able to subsist indefinitely on a diet that is markedly scorbutogenic to the guinea pig, but while doing so, stores in its tissues and excretes in its urine measurable amounts of ascorbic acid. Workers in this field are not in complete agreement regarding the relationship of the composition of the ingested diet to the amounts of ascorbic acid stored and excreted by the rat. Some investigators^{1,2} have contended that the composition of the diet is an influencing factor, while other investigators³⁻⁶ have failed to demonstrate that ascorbic acid output is affected by changes in dietary ingredients. Other investigators⁷⁻¹⁰ have contended that the ordinary constituents of the diet, such as sugar, fats and proteins, have no effect on the urinary excretion of ascorbic acid by the rat, but that high rates of excretion can be induced by feeding oats, oat oil, the unsaponifiable portion of oat oil, halibut liver oil and certain cyclic compounds of the terpene and sesqui-terpene series. Early reports by this group of investigators postulated the existence of a precursor from which the ascorbic acid was formed by the rat. In the later reports, however, these authors

* Authorized for publication on March 16, 1940, as paper No. 962 in the Journal Series of the Pennsylvania Agricultural Experiment Station.

¹ Hopkins, F. G., Slater, B. R., and Milliken, G. A., *Biochem. J.*, 1935, **29**, 2803.

² Menaker, M. H., January, 1938, Master's Thesis, Pennsylvania State College.

³ Svirbely, J. L., *Am. J. Physiol.*, 1936, **116**, 446.

⁴ Zilva, S. S., *Biochem. J.*, 1936, **30**, 857.

⁵ Scheunert, A., and Schieblich, M., *Z. Physiol. Chem.*, 1937, **247**, 272.

⁶ Mentzer, C., and Urbain, G., *Compt. Rend. Soc. Biol.*, 1938, **128**, 270.

⁷ Musulin, R. R., Tully, R. H., 3rd, Longenecker, H. E., and King, C. G., *Science*, 1938, **88**, 552.

⁸ Longenecker, H. E., Musulin, R. R., and King, C. G., *Proc. Am. Soc. Biol. Chem., J. Biol. Chem.*, 1939, **128**, p. lx.

⁹ Musulin, R. R., Tully, R. H., 3rd, Longenecker, H. E., and King, C. G., *J. Biol. Chem.*, 1939, **129**, 437.

¹⁰ Longenecker, H. E., Musulin, R. R., Tully, R. H., 3rd, and King, C. G., *J. Biol. Chem.*, 1939, **129**, 445.

abandon this view and postulate that the ascorbic acid is formed through intermediary metabolism, a view previously expressed by other investigators.⁶

Because of the conflicting reports concerning the possible origin of ascorbic acid in the body of the rat, as well as those concerning factors influencing its elimination, it seemed desirable that our previous studies be repeated, especially those relating to the carbohydrate portion of the diet. The present report contains some of the data obtained in the course of the latter investigation.

Experimental. In our studies we have used half-grown rats as the experimental subjects. In order to collect the urine quantitatively, the animals were maintained in individual, cylindrical, galvanized wire cages, each of which was suspended above a 10-inch glass funnel. Beneath the funnel was placed a small glass vessel containing, as a preservative, 4 ml of metaphosphoric acid solution (10%) to which had been added a trace of 8-hydroxy-quinolin and a one-fourth-inch layer of paraffin oil. The cages were provided with galvanized wire bottoms with mesh of sufficient size to allow all fecal particles to pass through. Under each cage was placed a finer galvanized wire screen to prevent the fecal matter from entering the funnel. In order to further minimize contaminants, which might enter the funnel, the cages were provided with special food cups and drinking fountains. Each cage and its supplementary equipment were cleaned thoroughly at weekly intervals and, when necessary, the funnels were changed daily.

The collections of urinary samples were begun as soon as the animals were transferred to the metabolism cages and fed the experimental diet. The 24-hour collections of urine were removed at a definite time each day, their volumes recorded and aliquots titrated with Na 2,6-dichlorobenzenone indophenol solution (175 mg of the dye in 500 ml of hot water).

The diets used in these studies were composed of fat-free casein 18, salt mixture 3, Cell U flour 2, fat-free yeast 8 and fat-free carbohydrate 77 parts. In the majority of experiments, the fat-soluble vitamins were furnished as beta carotene and calciferol. In a few instances these vitamins were supplied by adding 2 parts of cod liver oil to the basal diet. The carbohydrates used were: dextrinized corn starch, raw corn starch, sucrose, glucose, and in a limited number of feeding periods of short duration, mannose, sorbose, fructose and lactose. The amount of food consumed daily by each animal was recorded and the amount fed was only slightly in excess of that consumed during the previous 24 hours.

In the instance of the first series of animals, all animals were fed the diet containing the dextrinized corn starch during the first period. This was followed by the sucrose-containing diet, the glucose-containing diet and the starch-containing diet, respectively. At the termination of the studies with these diets, the animals were again fed the dextrinized corn starch diet for a period of several weeks.

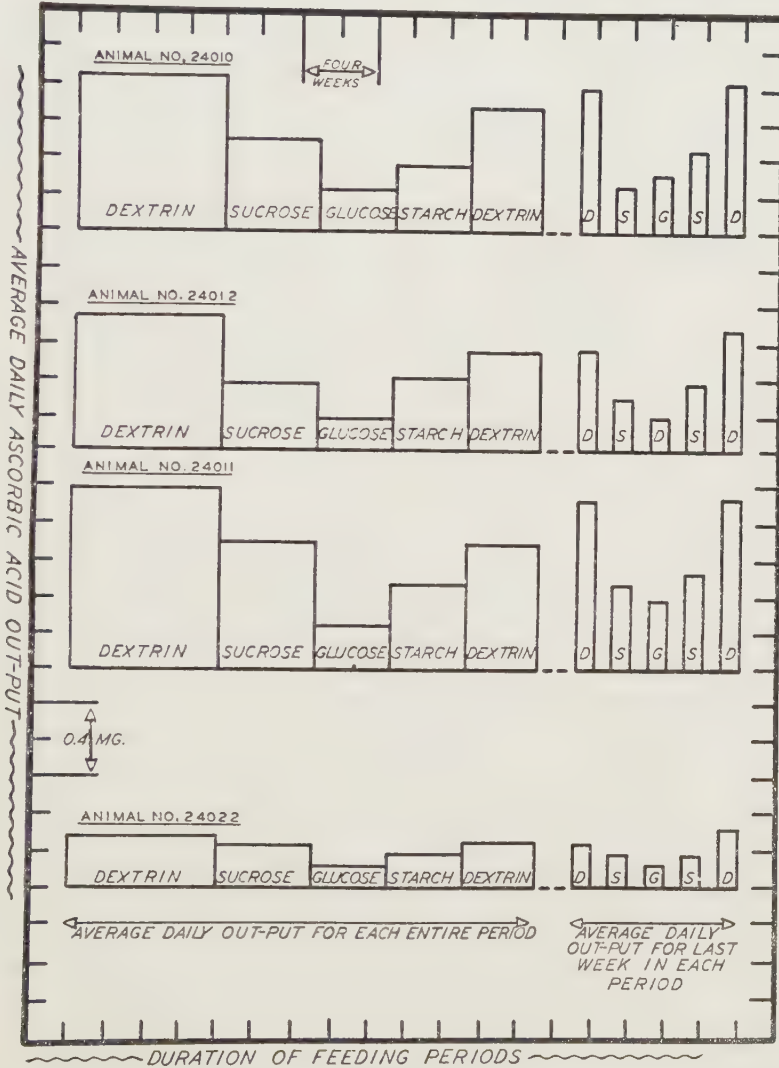


FIG. 1.

Amounts of ascorbic acid eliminated in the urine of rats while receiving diets similar in composition, the only difference being in the type of carbohydrate which they contained.

With subsequent series of animals, diets of similar composition were used, the experimental difference being in the sequence in which they were fed to the test animals.

Since space does not permit the presentation of all the data at this time, only the condensed data relating to the urinary ascorbic acid output of 4 typical animals from the first series are given

The duration of the various feeding periods were 8, 5, 4, 4 and 4 weeks, respectively. The urinary ascorbic acid elimination has been expressed as the average daily output for the entire period during which each of the respective diets was fed. In order to indicate the probable carry-over effect of one diet on the succeeding diet, the average daily urinary ascorbic acid output for the last week of each feeding period is given on the right of the graph (Fig. 1).

Discussion. With all of the animals used, the urinary ascorbic acid output was greatest while the animals were consuming the diet containing the dextrinized corn starch. This was found to be true irrespective of the sequence in which the diets were fed. On the other hand, in most instances, these same animals eliminated the smallest quantity of ascorbic acid while consuming the glucose-containing diet. However, the frequency of the latter observation seemed to depend somewhat on the sequence in which the glucose diet was fed.

The greater ascorbic acid elimination resulting from the consumption of the diet containing the dextrinized corn starch as compared to the other diets, does not appear to be readily explainable from the data at hand. While the average daily consumption of the dextrinized corn starch was slightly greater than the consumption of the other diets and while there seemed to be a slight correlation between daily food intake and the amount of urinary ascorbic acid, this observation is sufficient only to explain a small portion of the increase in ascorbic acid elimination. The inadequacy of such an explanation can be readily observed from the fact that animal No. 24022 consumed as much of the respective diets as did animal No. 24011 and, while doing so, eliminated only about one-third as much urinary ascorbic acid as did the latter animal. However, it may be stated that the relative amounts of ascorbic acid eliminated by the various animals while receiving the sequence of diets were of the same order of magnitude for the respective diets.

The question as to why greater amounts of ascorbic acid were excreted, while the rats were consuming the dextrinized corn starch diet, than when the raw corn starch diet was consumed, cannot be

answered at this time. The possibility of the existence of different amounts of an ascorbic acid precursor in the two diets does not seem tenable, at least in this instance. To verify this point, some of the rats, after having been returned to the dextrinized corn starch diet for several weeks, were given weighed amounts (25, 50 or 100 mg) of carvone (Eastman No. 1094) to determine the effect of this substance on ascorbic acid output. Since it was found impossible to determine, quantitatively, the amount of this volatile substance actually consumed by the rats when it was mixed with the diet, the carvone was diluted with olive oil and given by stomach tube. However, with this procedure, the tests proved unsatisfactory in that the animals began to lose weight after the administration of the first or the second dose of carvone and the majority of the test animals died within 6 or 7 days. In no case was there more than a twofold increase in the amount of ascorbic acid eliminated in any one day and such increases did not remain consistent from day to day.

Conclusions. It is apparent that the amount of ascorbic acid eliminated in the urine of the rat depends upon at least 2 major factors, namely, the type of carbohydrate ingested by the rat, and the physiological variations within the rat itself. Since it was frequently found that ascorbic acid elimination varied as much with different animals as it did with different diets, it is evident that the latter factor must be given due consideration. The data submitted, however, do not explain the origin of the ascorbic acid nor do they explain the differences in the amounts of ascorbic acid excreted in the urine of different animals while receiving comparable amounts of the same diet. A number of theoretical possibilities suggest themselves but these offer no immediate solution to the problem. It would serve no useful purpose to say that the ascorbic acid is probably of endogenous origin or that it has its origin in intermediate metabolites. Such suggestions fall far short of explaining the origin of the ascorbic acid in the body of the animal and would be equally ineffective in explaining why different animals excreted different amounts of this substance while consuming comparable portions of the same diet.

Loss of Carbohydrate Metabolism Factor During Boiling of Vegetables.*

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Baltimore, Md.*

Abnormally high respiratory quotients during carbohydrate assimilation are given by rats that have been maintained for a number of months on a fat-deficient diet.^{1,2} These high quotients, a large proportion of them above 1, have been attributed to a lack, in the fat-deficient diet, of an accessory factor that is necessary for normal carbohydrate metabolism.^{1,2}

In the present paper it will be shown that rats maintained for 3 months or longer on a diet of boiled vegetables give carbohydrate RQ's that are abnormally high as compared with animals on a stock diet. This is interpreted as indicating that the boiled vegetable diet is likewise deficient in this carbohydrate metabolism factor. As it has previously been shown^{1,†} that raw vegetables and fruits apparently possess a high content of this factor, it now becomes evident that a loss of this factor takes place during the boiling of vegetables. That this is the case is confirmed by feeding rats that show the abnormal carbohydrate metabolism the same vegetable diet, but autoclaved instead of boiled. The effect of the autoclaved diet on these rats will be shown to be similar to that obtained by feeding the ether-soluble substances extracted from raw vegetables and animal fats in previous investigations.^{1,2,†} namely, the restoration of the carbohydrate assimilatory RQ's to nearly normal.

Diets. Boiled vegetable diet. A minced mixture of 2 parts by weight of potatoes, 1 part of carrots, and 1 part of string beans, to which was added 0.25% NaCl and a surplus of tap water, was

* Presented in abstract form before the American Society of Biological Chemists, at Baltimore, April 2, 1938 (*J. Biol. Chem.*, 1938, **123**, cxxv).

¹ Wesson, L. G., *J. Biol. Chem.*, 1927, **73**, 507.

² Wesson, L. G., and Burr, G. O., *J. Biol. Chem.*, 1931, **91**, 525; Wesson, L. G., *J. Biol. Chem.*, 1933, **100**, 365; Wesson, L. G., and Murrell, F. C., *J. Biol. Chem.*, 1933, **102**, 303; *Proc. Soc. Exp. Biol. and Med.*, 1934, **31**, 1118.

† Previously unpublished data supporting this statement: Using a group of 4 rats in each case, the average maximum carbohydrate assimilatory RQ before dosing with approximately 0.3 g of the ether-soluble substances of raw potato was 1.08, after dosing it was 0.97; of raw carrot, 1.05 before dosing, and 0.89 after dosing; of raw apple, 1.05 before, and 1.01 after dosing.

actively boiled, with frequent stirring, for 8 hr in an open saucepan. At the end of that time, the mixture was of a pasty consistency, and of somewhat less than the original volume. Before the mixture cooled, 10 ml of an aqueous 5% solution of Na benzoate per 500 ml (0.1%) was stirred into it. It was then refrigerated until fed to the rats. In addition to the boiled vegetable mixture *ad libitum*, approximately 0.5 g of ether-extracted brewers' yeast per rat was fed daily during most of the work.

Autoclaved vegetable diet. The same vegetable mixture that was used in the preparation of the boiled vegetable diet, without the addition of water, was heated in sealed jars for 4 hr at 15 lb steam pressure.

Procedures. The respiratory exchange was determined by the use of a closed-circuit calorimeter of the type previously employed for this purpose.³ The dextrin test meal following 18 to 20 hr of fasting was the same as that described in previous papers on this subject.^{2,3}

Results. Six male and 5 female rats, 6 months old, were used. Approximately 3 months' feeding of the boiled vegetable diet was required before the first indication of abnormality in the carbohydrate metabolism was observed. This abnormality is shown in Table I by a significant difference between the carbohydrate assimilatory RQ's of these 11 rats and the corresponding RQ's of rats on a stock diet. The high RQ's are distinctly abnormal and are similar to those of rats on a fat-deficient, purified diet.²

In Table II are given the average carbohydrate RQ's of 6 of the abnormal rats before and after 7 days' feeding of the autoclaved diet. These values show that a marked lowering of the RQ's is

TABLE I.
RQ's Following Dextrin Test Meals with Rats on a Stock Ration and on a Boiled Vegetable Diet.

Time after test meal hr	Stock ration ³ 10 runs on 10 rats			Boiled vegetable diet 82 runs on 11 rats		
	Mean RQ	RQ's > 1.00 No. Max.		Mean RQ	RQ's > 1.00 No. Max.	
1.5	.90 ± .06*	0	1.00	1.00 ± .005	31	1.09
2.5	.91 ± .02	0	1.00	1.01 ± .004	50	1.09
3.5	.89 ± .02	0	0.99	1.03 ± .005	59	1.11
4.5	.88 ± .02	0	1.00	1.01 ± .007	58	1.11
5.5	.85 ± .02	0	1.00	0.94 ± .009	17	1.04

*Standard deviation of the mean.

³ Wesson, L. G., *J. Nutr.*, 1931, **3**, 503.

TABLE II.
Lowering of Abnormal Dextrin RQ's of 6 Rats on a Boiled Vegetable Diet, Followed by 7 Days' Feeding of the Autoclaved Diet.

Time after test meal hr	Boiled vegetable diet 6 runs on 6 rats			Autoclaved vegetable diet 6 runs on 6 rats		
	Mean RQ	RQ's > 1.00 No. Max.		Mean RQ	RQ's > 1.00 No. Max.	
1.5	1.02 ± .02	3	1.08	.93 ± .02	1	1.01
2.5	1.02 ± .01	5	1.07	.94 ± .02	0	0.98
3.5	1.07 ± .01	6	1.11	.95 ± .01	0	0.98
4.5	1.01 ± .02	5	1.04	.97 ± .02	1	1.01
5.5	0.90 ± .02	0	0.99	.94 ± .01	0	0.98

produced by the autoclaved diet, similar to the effect of animal fats and of the ether-soluble substances of raw vegetables and fruit.^{1, 2, †}

Conclusions. A loss of the carbohydrate metabolism factor takes place during the boiling of vegetables in an open vessel. This makes plausible the possibility that some deficiency of this factor occurs in many human dietaries. Since its lack causes abnormal fat formation and a disturbance of the carbohydrate metabolism of rats, it seems reasonable to consider a possible dietary deficiency of this factor in the study of prediabetic obesity.

Summary. Abnormally high respiratory quotients during carbohydrate assimilation are given by rats that have been maintained on a diet of boiled vegetables. This indicates a loss of an appreciable part of the carbohydrate metabolism factor which the vegetables contain in the unboiled condition.

The question is raised as to a possible causative connection of a dietary deficiency of this factor with prediabetic obesity.

Antistreptolysin Values in the General Population of Puerto Rico.

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While studying the antistreptolysin content of the blood in cases of recurrent tropical lymphangitis¹ we were strongly impressed by the difference in values shown by these and by apparently normal cases. The question came up as to what should be considered as normal value for the general population under our local conditions.

Coburn and Pauli² determined the natural level of antistreptolysin in human blood, selecting for study a group of student nurses entering training at the Presbyterian Hospital, New York, on September 1932. These subjects were kept under clinical observation. Throat cultures were taken during periods of respiratory infection and antistreptolysin determinations were made at different intervals. The findings were classified in several groups: (a) those who contracted pharyngitis due to hemolytic streptococcus infection, (b) those who contracted other infections, the agent being unknown, (c) those who appeared to escape infection with hemolytic streptococci.

At the beginning of their study the median value for 30 individuals was 63 units. Only 5 subjects had a titer higher than 100 units. Ten subjects infected with hemolytic streptococci showed a subsequent rise in titer. None of those who escaped infection developed a significant rise in titer. The median titer of the group that escaped infection was 50 units 18 months later. These observations were interpreted by the authors to mean that the natural human antistreptolysin value is ordinarily about 50 units.

In order to determine the antistreptolysin values in our general population we proceeded to study a number of individuals, making the following classification: (1) apparently normal throats with no history of streptococcus infection and from which hemolytic streptococci could not be cultured at the time the determination was made, (2) apparently normal throats from which hemolytic streptococci were cultivated, and (3) apparently normal throats with a definite

¹ Morales-Otero, P., and Pomales-Lebrón, A., *P. E. J. Pub. Health and Trop. Med.*, 1936, **12**, 43.

² Coburn, A. F., and Pauli, R. H., *J. Exp. Med.*, 1935, **62**, 129.

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history of previous streptococcus infection from which hemolytic streptococci could not be cultured.

Material and Methods. The material and methods used in this work were the same as those previously employed by us.¹ The streptolysin was standardized, using standard Todd's globulin kindly supplied to us by Dr. Coburn. Single determinations were made in each case. Throat cultures and blood specimens were taken at the same time.

Results. One hundred and thirty-six determinations were made on Group 1. The lowest value was 18 units and the highest 150, with an average of 84.1 units.

Forty-three determinations were made on Group 2.* The lowest determination in this group was 48 units and the highest 350, with an average of 144.9 units.

Ninety-six determinations were made on Group 3. The lowest determination in this group was 37 units and the highest 333, with an average of 154.5 units.

Summary. Two hundred and seventy-five antistreptolysin determinations were made in apparently normal persons from the general

TABLE I.
Antistreptolysin Values in the General Population of Puerto Rico.

Antistreptolysin titer	No. of individuals	Group 1	Group 2	Group 3
0-20	1	1	0	0
21-40	9	8	0	1
41-60	40	31	4	5
61-80	34	28	2	4
81-100	43	32	4	7
101-120	30	9	8	13
121-140	38	18	6	14
141-160	32	9	7	16
161-180	12	0	2	10
181-200	12	0	2	10
201-250	13	0	4	9
251-300	6	0	2	4
301-400	4	0	2	2
401-500	1	0	0	1
Mean antistreptolysin titer by groups		84.1 units	144.9 units	154.5 units
Mean titer for the 3 groups:		127.8 units.		

Group 1—Consists of persons of apparently normal throats with no history of streptococcus infection and from which hemolytic streptococci could not be cultured.

Group 2—Consists of persons of apparently normal throats from which hemolytic streptococci were cultivated.

Group 3—Consists of persons of apparently normal throats with a definite history of previous streptococcus infection from which hemolytic streptococci could not be cultured.

* Strains belong to Groups A, C and G.

population of Puerto Rico. The mean value of 136 apparently normal persons from which hemolytic streptococci could not be cultured at the time the determination was made was 84.1 units. In 43 apparently normal subjects harboring hemolytic streptococci at the time the determination was made, the mean antistreptolysin value was 144.9 units. In 96 normal persons, not harboring demonstrable hemolytic streptococci at the time the determination was made, but having a definite past history of streptococcus infection, the mean value was 154.5 units. The mean value for the 3 groups was 127.8 units.

11404 P

Effects of Renin and of Angiotonin Upon Isolated Perfused Heart.

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The authors have examined the influence of renin, and of angiotonin, upon the isolated hearts of cats perfused with Ringer-Locke solution by the Langendorff method. Renin was prepared by alcohol precipitation of fresh pig's kidney cortex and fractional precipitation with ammonium sulphate, followed by prolonged dialysis. Angiotonin was prepared by the method of Page† and Helmer.¹ The pH of this solution was adjusted to 7.0 with dilute sodium hydroxide. It was injected, in doses shown to produce minimal to large pressor effects in intact animals, into the stream of the perfusate just above the heart.

Renin. The observations of Tigerstedt and Bergman² and of Hessel³ that renin is without influence upon the isolated heart, were entirely confirmed by 33 injections in 16 experiments.

Angiotonin. Coronary Flow. Twenty-four injections in 12

* Working under the Jacques Loeb and Archibald Fellowships.

† The authors are indebted to Dr. Page for his kindness in furnishing a quantity of angiotonin for comparison with that prepared by us.

¹ Page, I. H., and Helmer, O. M., *J. Exp. Med.*, 1940, **71**, 29.

² Tigerstedt, R., and Bergman, P. G., *Skand. Arch. Physiol.*, 1898, **8**, 223.

³ Hessel, G., *Klin. Wchnschn.*, 1938, **17**, 843.

experiments reduced the coronary output by from 27% to 86% (average 45%) in the presence of normal sinus rhythm. Four injections in one preparation were without result. The effect was maximal within 30 to 75 seconds after the beginning of the injection and lasted for one to 6 minutes. It showed no tendency to diminish with successive injections, though not more than 4 were administered to any one preparation. Four injections in 3 preparations, in which ventricular fibrillation was maintained by faradic stimulation of the ventricle, brought about a decrease in coronary flow by from 16% to 48% (average 28%). A late rise in coronary flow (averaging 16% for all injections) was frequently recorded. This late increase in flow was measurably reduced with successive injections.

Amplitude of beat. The amplitude of ventricular contraction was increased following every injection by from 18% to 300% (average 92%). This commenced later than the slowing of coronary flow, reached its maximum in 1 to 3 minutes, and persisted for 3 to 15 minutes. The effect of angiotonin upon amplitude of beat showed no consistent tendency to diminish with successive injections.

Heart Rate. Significant effects upon the heart rate were not recorded. In some experiments the rate of beat diminished slightly during the period of reduced coronary flow.

Summary. Upon the isolated hearts of cats perfused with Ringer-Locke solution renin produced no significant effect. Angiotonin on the other hand brought about decrease in coronary flow and increase in amplitude of beat, but no consistent effect upon heart rate.

11405 P

Pituitary Function in Parabiotic Triplet Rats.

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Rat ovaries that have been made to function under the stimulus of the male pituitary as in the experiments of Goodman¹ (ovarian grafts), Witschi and Levine² (parabiosis) and Pfeiffer³ (mascu-

¹ Goodman, L., *Anat. Rec.*, 1934, **59**, 223.

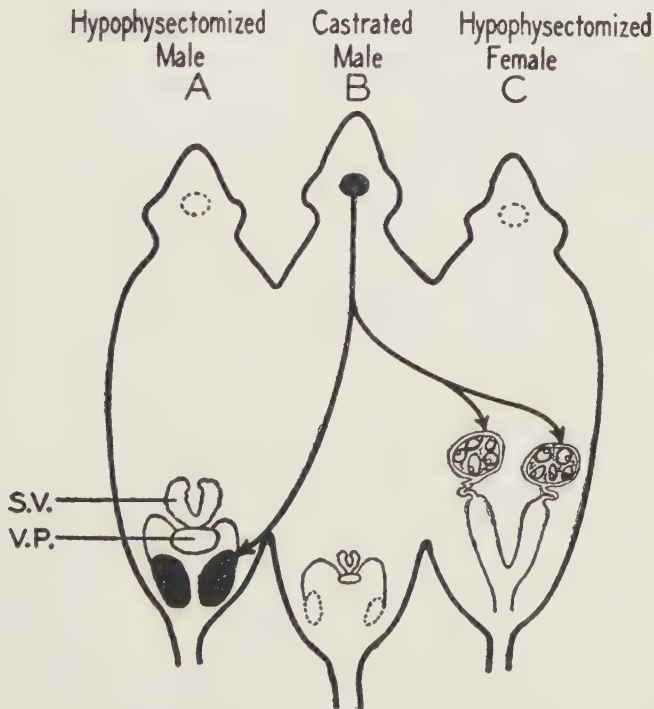
² Witschi, E., and Levine, W. T., *Proc. Soc. Exp. Biol. and Med.*, 1934, **32**, 101.

³ Pfeiffer, C. A., *Am. J. Anat.*, 1936, **58**, 195.

linized female pituitaries) have uniformly failed to become luteinized. The luteinizing hormone, however, has been shown to be present in small amounts in the pituitaries of normal male rats and abundant in those of castrated animals (Hellbaum and Greep⁴). Cutuly, *et al.*,⁵ have shown that the pituitary of a castrated male stimulates the interstitial cells as well as the seminiferous tubules of a parabiotic hypophysectomized male partner. Furthermore, there is considerable experimental evidence for assuming that the luteinizing hormone is identical with the substance responsible for the internal secretory function of the male gonad. The available evidence then is contradictory in that the male pituitary appears to secrete luteinizing hormone if the end organ is a male gonad, and *only* follicle-stimulating hormone if the end organ is a female gonad.

The present experiments were devised to bring the entire repro-

FIGURE I



⁴ Hellbaum, A. A., and Greep, R. O., *Am. J. Anat.*, 1940, in press.

⁵ Cutuly, E., McCullagh, D. R., and Cutuly, E. C., *Endocrinology*, 1937, **21**, 241.

ductive systems of each sex under the simultaneous influence of a single male pituitary.

Male rats, aged 21 days, were gonadectomized and joined in parabiosis with 2 littermates—a male on one side and a female on the other. (See also Fig. 1 drawn like that of DuShane and others.⁶) Four to 6 days later the 2 outside triplets were hypophysectomized. Autopsies were performed 7 to 16 days after hypophysectomy. The gonads, sexual accessories, thyroids and adrenal glands were weighed and examined grossly. Vaginal smears were followed in some instances. The observations reported are based on 8 sets of triplet parabionts in which all except the central animal had been completely hypophysectomized.

On the 7th day following the joining of these animals the vaginas of the hypophysectomized females opened and rapid testicular growth in the hypophysectomized male became apparent. The autopsy findings, illustrated by the data from a typical experiment (Table I), show that the principal physiological action of the castrated male pituitary on the ovary of a hypophysectomized female triplet parabiont is to promote the growth of follicles and cause the continuous secretion of estrogen as attested by persistent vaginal cornification and a distended uterus. The ovaries have not yet been examined microscopically for the presence of interstitial-cell or luteal stimulation but there was no evidence of luteinization by gross inspection. The hypophysectomized male parabiont, which had derived its gonadotropic stimulus from the same pituitary gland that produced only follicle growth in the female, showed a marked stimulation of the testes, and the secondary sexual structures, such as the prostate and seminal vesicles, were greatly enlarged.

The secondary sexual structures of the central castrate remained entirely atrophic in all cases.

The peculiar ability of the castrated male rat pituitary to evoke

TABLE I.
Organ Weights of 3 Animals Which Had Been Joined Together for 19 days. Rats "A" and "C" Had Been Hypophysectomized for 13 Days and the Central Animal "B" Had Been Castrated Since the Start of the Experiment.

Rat	Body wt at death, gm	Testes, mg	Seminal vesicles, mg	Ventral prostate, mg	Ovaries, mg	Empty uterus, mg	Adrenals, mg	Thyroid, mg
A	52	1036	54	106			6.6	4.45
B	67		8	7			20.5	7.60
C	52				44.4	286	9.6	6.34

⁶ DuShane, G. P., Levine, W. T., Pfeiffer, C. A., and Witschi, E., *PROC. SOC. EXP. BIOL. AND MED.*, 1935, **33**, 339.

a follicular response in a female and at the same time stimulate the testes of a male to secretory function is difficult to interpret considering the results which have been obtained with purified follicle-stimulating extracts of the pituitary.^{7, 8, 9} Several explanations suggest themselves but all are purely conjectural: (a) the threshold for luteinization of the ovary may be far above that necessary to produce stimulation of testicular interstitial cells; (b) possibly the interstitial cell stimulating and luteinizing hormones are not identical; (c) it may be extremely difficult to cause luteinization of follicles which grow rapidly and become cystic.

11406 P

Efficacy of Pellets of Posterior Hypophysis and of Pitressin in Oil in Diabetes Insipidus.

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The efficacy of the subcutaneous administration of dried posterior pituitary gland in diabetes insipidus has not been reported previously. Pellets of this material have been prepared and implanted subcutaneously into 4 cats with experimentally produced diabetes insipidus* and into 2 patients with diabetes insipidus. The results are shown in Table I. It is to be noted that mixing the material with tyrosine or impregnating the pellet with beeswax did not prolong the effect in the cats. An inflammatory reaction which occurred at the site of implantation of sterile pellets in the patients later required drainage. An attempt was made to prolong the action in man by impregnation of the pellets with lanolin or beeswax. The reaction which developed at the site of implantation was so severe that the pellets had to be removed before complete absorption occurred.

⁷ Greep, R. O., and Fevold, H. L., *Endocrinology*, 1937, **21**, 111.

⁸ Greep, R. O., *PROC. SOC. EXP. BIOL. AND MED.*, 1939, **42**, 454.

⁹ Chow, B. F., Greep, R. O., and van Dyke, H. B., *J. Endocrinology*, 1940, **1**, 439.

* Available through the courtesy of W. R. Ingram, Department of Anatomy, State University of Iowa, College of Medicine.

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TABLE I.
Duration of Effect of Dried Posterior Pituitary Gland Implanted Subcutaneously
in Cats and in Man with Diabetes Insipidus.

Control period					Posterior pituitary pellets				
	No. Days	Avg fluid intake, cc	Avg urinary output, cc	Avg urinary specific gravity	Wt of pellets, mg	Days active	Avg fluid intake, cc	Avg urinary output, cc	Avg urinary specific gravity
Cat	1	14	261	1.016	102.0	11		151	1.030
"	2	6	580	1.006	98.0	6		231	1.019
"	3	7	488	1.008	190.0*	4		316	1.015
"	4	7	370	1.012	100.0†	7		175	1.025
Patient	1	3	13,170	1.000	300.0	3	2,650	2,025	1.015
"	2	4	11,300	1.000	300.0	3	1,400	1,283	1.015

*Pellet consisted of equal parts of tyrosine and dried posterior pituitary gland.

†Pellet impregnated with beeswax.

The above results demonstrate that pellets of dried posterior pituitary gland implanted subcutaneously control the manifestations of diabetes insipidus, but that this method is not applicable for treatment in man. For this reason pitressin tannate† in oil was employed. This material was administered to 3 cats with experimentally produced diabetes insipidus and to 3 patients with the syndrome. In the cats 1.0 cc ameliorated the manifestations for 3 to 7 days and in man for 30 to 82 hours. There were no unpleasant or deleterious general or local reactions. The symptoms of the disease have been controlled in the 3 patients by the subcutaneous injection of 1.0 cc every 36 to 57 hours.

† Supplied through the courtesy of Parke, Davis and Company.

11407 P

Rôle of the Sympathetic Nervous System in Experimental Neurogenic Hypertension.

KEITH S. GRIMSON. (Introduced by Dallas B. Phemister.)

From the Department of Surgery, University of Chicago.

Recent clinical studies¹ have renewed interest in central or psychosomatic factors in essential hypertension. The sustained neurogenic hypertension in dogs described by Heymans and Bouckaert² seems from this point of view to afford a better experimental approach to the problem of hypertension and sympathectomy than the renal hypertension described by Goldblatt,³ and shown to be uninfluenced by total sympathectomy.⁴⁻⁷ The recent demonstration, Grimson, Bouckaert and Heymans,⁸ that a sustained neurogenic hypertension of renal origin may be produced by a central reflex mechanism tends to correlate these two methods for producing experimental hypertension. The present study is based upon an effort to determine the blood pressure levels produced by section of the depressor nerves in normal dogs and compare them with the pressure levels produced by the same procedure in dogs sympathectomized with the exception of the nerve supply to the kidneys and adrenals, as well as to study the effects of renal denervation, splanchnic resection, and total paravertebral sympathectomy on the former group.

Heymans and Bouckaert⁹ have shown that section of the depressor nerves produces a persistent hypertension and that total sympathectomy eliminates the hypertension. Goldblatt, Kahn, Bayless and Simon¹⁰ have recently failed to obtain this type of hypertension

¹ Katz, L. N., and Leiter, Louis, *Psychosomatic Med.*, 1931, **1**, 101.

² Heymans, C., and Bouckaert, J. J., *C. R. Soc. Biol.*, 1931, **106**, 471; *Bull. Acad. Roy. Med. de Belg.*, 1939, p. 441.

³ Goldblatt, H., Lynch, J., Hanzal, R. F., and Summerville, W. W., *J. Exp. Med.*, 1934, **59**, 347.

⁴ Alpert, L. F., Alving, A. S., and Grimson, K. S., *PROC. SOC. EXP. BIOL. AND MED.*, 1937, **37**, 1.

⁵ Freeman, N. F., and Page, I. H., *Am. Heart J.*, 1937, **14**, 405.

⁶ Heymans, C., Bouckaert, J. J., Bayless, F., and Samaan, A., *C. R. Soc. Biol.*, 1937, **126**, 434.

⁷ Verney, E. B., and Vogt, M., *Quart. J. Exp. Physiol.*, 1938, **28**, 253.

⁸ Grimson, K. S., Bouckaert, J. J., and Heymans, C., *PROC. SOC. EXP. BIOL. AND MED.*, 1939, **42**, 225.

⁹ Heymans, C., and Bouckaert, J. J., *C. R. Soc. Biol.*, 1935, **120**, 82.

¹⁰ Goldblatt, H., Kahn, J. R., Bayless, F., and Simon, M. A., *J. Exp. Med.*, 1940, **71**, 175.

and Nowak and Walker¹¹ have stated that some hypertension follows depressor nerve section in sympathectomized dogs. These contraindications have further stimulated this study.

In 9 dogs both carotid sinuses were excised, the left vago-sympathetic-depressor nerve was cut, and a segment of the right sympathetic depressor trunk was removed. The control blood pressures were respectively 131, 133, 134, 134, 138, 138, 144, 148 and 174. These dogs were observed from 16 to 163 days after modulator nerve section and their pressure readings averaged respectively 238, 246, 239, 212, 194, 256, 257, 214 and 226. The late readings in 3 animals were appreciably higher than the early readings. Three animals had occasional readings of 280 to 300. None of the dogs failed to develop a hypertension and in none of them was there any late lowering of the hypertension.

In 6 normal dogs both paravertebral sympathetic chains were removed. Their control blood pressures averaged 142 and their pressures 14 to 29 days after sympathectomy averaged 112. Because of the observation (Grimson, Wilson and Phemister¹²) that sympathectomized dogs recover in a few months a new central vasomotor mechanism and restore their preoperative blood pressure the modulator nerves in these dogs were sectioned in the manner described above 14 to 29 days after the sympathectomy. Two dogs died shortly after operation without pressure elevation. The other 4 during the next 30 days had an average pressure of 98. No elevation was observed. Two were observed 90 and 108 days and developed pressures higher than before the sympathectomy, 164-206. Two other dogs sympathectomized 26 and 28 months previously and with restoration of their blood pressure to averages of 148 and 152 developed average pressures after modulator nerve section of 169 and 176 mm respectively with occasional readings of 200. This observation of levels higher than before sympathectomy suggests that the recovered central vasoconstrictor mechanism¹² is influenced by the modulator nerves.

Seven dogs* have now been sympathectomized with the exception of the splanchnic supply to the adrenals and kidneys according to the described technic.⁸ Their blood pressures just preceding modulator nerve section averaged 136 and during several weeks afterward 195. This elevation is definitely less than the average of the nine normal dogs with modulator nerve section described above which

¹¹ Nowak, S. J. G., and Walker, I. J., *New England J. Med.*, 1939, **220**, 269.

¹² Grimson, K. S., Wilson, H., and Phemister, D. B., *Ann. Surg.*, 1937, **106**, 801.

* Three observed in Prof. C. Heyman's laboratory in Ghent.

was 231. Renal denervation in four of these 7 dogs has restored their pressure to about the normal level.

Further experiments have shown that renal denervation alone neither prevents nor appreciably alters the hypertension produced in normal dogs by modulator nerve section. They have also confirmed the observations of Nowak and Walker¹¹ that abdominal sympathectomy and division of the splanchnic nerves as well as complete sympathectomy except for one thoracic chain fails to restore the blood pressure of neurogenic hypertensive dogs to normal. Total sympathectomy as described above lowered the pressure of 3 neurogenic hypertension dogs from 239, 226 and 246 to 101, 122 and 91 respectively during 30, 18, and 40 days of observation. After 30 and 40 days in 2 of these animals blood pressure recovery was evident and progressed toward a moderate hypertension level. This parallels but exceeds the recovery following paravertebral sympathectomy in normal dogs previously reported.¹²

11408 P

Renal Phosphatase in Experimental Nephropathies.*

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The specific function of the rich phosphatase content of the kidney is still unknown. Since the kidney is almost invariably involved in metastatic calcification and is often the site of pathologic calcification it seemed possible that by comparing the location of the deposits of lime salts in these conditions with that of the phosphatase something might be learned concerning the relation of this enzyme to renal function.

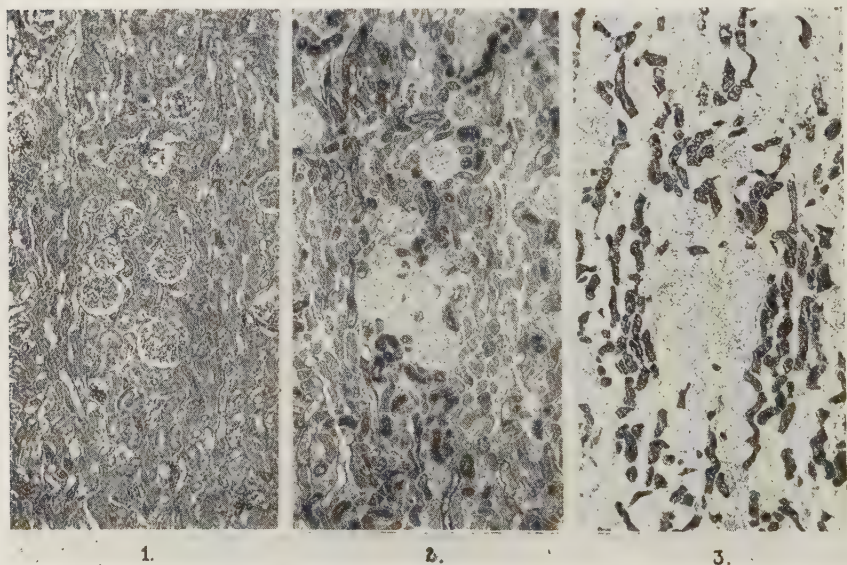
For this purpose we studied phosphatase, acting optimally at a pH of about 9.0 on sodium glycerophosphate, in the kidneys of normal dogs and of dogs in which a toxic nephrosis has been produced by uranium nitrate, potassium bichromate and bichloride of mercury. We compared sections stained for phosphatase by Gomori's¹ method with the quantity of the enzyme obtained in aqueous extracts of the cortical tissue of the same kidneys as determined by Bodansky's

* Aided by a grant from the Committee on Therapeutic Research of the Council on Pharmacy and Chemistry of the American Medical Association.

¹ Gomori, G., *Proc. Soc. Exp. Biol. and Med.*, 1939, **42**, 23.

chemical method. We soon found, however, that in kidneys in which pathologic calcification was present, there was no correlation between the quantity of phosphatase revealed by the chemical method and the microscopic picture in the Gomori-stained sections.

We therefore stained 3 consecutive sections of kidneys of the dogs used in these experiments, one with routine hematoxylin and eosin, one with Gomori's¹ stain for phosphatase and one for calcium phosphate only by von Kossa's method. The routine sections revealed the location of necrotic or otherwise damaged tubular epithelium. Sections stained by Gomori's method showed all of the calcium phosphate present in approximately quantitative relations. Normally, phosphatase is present in the marginal zone, next the lumen, of the epithelium lining the proximal convoluted tubules. It is most abundant in the first two-thirds or three-fourths of these tubules, that is, in the labyrinth; less abundant in the straight terminal portion, that is, in the medullary rays and in the outer stripe of the outer zone of the medulla. In Gomori-stained sections the calcium was more abundant and stained more deeply where it had been precipitated in normal or only slightly damaged cells by the action of the phosphatase during the process of staining than in those regions where its presence was the result of pathologic changes. Sections stained



K Hg 6.

1. H. & E. 2. Gomori. 3. Kossa. One injection—3 mg. HgCl_2 per 100 cc blood. Died 78 hours later.

by von Kossa's method revealed only that preformed calcium phosphate which was the result of pathologic calcification. In our experiments, this was present chiefly in the medullary rays in amounts varying with the length of survival of the animal, while the labyrinth was either free or showed only minute amounts (Fig. 1).

Calcification was found to occur early (28 hours after injection) and abundantly in the kidneys of dogs given a single intravenous injection of bichloride of mercury equal to 3 mg per 100 cc of blood. This dose of bichloride causes very severe injury to the epithelium of the straight distal portion of the proximal convoluted tubules, but no visible injury to the glomeruli. Doses of potassium bichromate and uranium nitrate that induced marked necrosis of the tubular epithelium have not been found to induce pathologic calcification in the kidneys of animals that have survived for 3 days.

None of the poisons (uranium, bichromate, bichloride of mercury) in the doses used appear to inactivate the phosphatase although they may kill the cell that contains it. This conclusion is confirmed by the results of quantitative determinations of the phosphatase in these kidneys by Bodansky's method and by study of stained sections. Necrotic and desquamated tubular epithelium of kidneys of dogs poisoned by bichloride stains diffusely by Gomori's method instead of deeply along the luminal margin. Granular material stained by Gomori's but not by von Kossa's method in the capsular space of some glomeruli is continuous with similar material in the tubule which takes origin from such a glomerulus. It is believed to be cellular debris containing active phosphatase which has been regurgitated into the capsular space from the damaged tubule. Cellular debris accumulated in the straight terminal portion of the proximal convoluted tubules above the narrow part of Henle's loop stained both by Gomori's and von Kossa's method, and appears, therefore, to contain both active phosphatase and preformed calcium phosphate.

Metabolism of Free Citric Acid in the Rat.*

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Studies in which pure citric acid was given by mouth to human subjects have shown that citric acid may be almost completely metabolized.¹⁻⁵ In the dog, large amounts of orally administered citric acid are likewise destroyed, less than 1% of the compound given appearing in the urine and no "extra" citric acid in the feces.⁶ Similar results have been obtained with rabbits⁷ and swine.^{8, 9} The possibility that the citric acid administered was not absorbed was precluded by Langecker⁷ who found a prompt and prolonged rise in the level of blood citrate and showed further that the enzymes and bacteria present in the intestinal tract of the rabbit do not destroy citric acid even when incubated for as long as 9 hours. The following experiments were conducted in order to determine whether or not the rat also possesses the ability to metabolize free citric acid.

A series of rats maintained in metabolism cages was fed the following diet *ad libitum*: lactalbumin (Borden No. 15-42) 15.0, dextrin 50.5, hydrogenated fat (Crisco) 27.0, cod liver oil (Mead) 5.0, and salt mixture¹⁰ 2.5%. This diet was supplemented daily with 2 cc of a solution containing 80 mg of liver extract (Lilly 343) and 200 mg of Ryzamin-B (Burroughs Wellcome).

The cages were placed over large glass funnels containing a 3/16

* The data herein presented are taken from a dissertation submitted by Carl A. Kuether in partial fulfillment of the requirements for the degree of Master of Science in Wayne University, 1940.

This investigation has been made with the assistance of a grant from the Committee on Therapeutic Research, Council on Pharmacy and Chemistry, American Medical Association.

¹ Östberg, O., *Skand. Arch. f. Physiol.*, 1931, **62**, 81.

² Kuyper, A. C., and Mattill, H. A., *J. Biol. Chem.*, 1933, **103**, 51.

³ Boothby, W. M., and Adams, M., *Am. J. Physiol.*, 1934, **107**, 471.

⁴ Gonce, J. E., and Templeton, H. L., *Am. J. Dis. Child.*, 1930, **39**, 265.

⁵ Schuck, C., *J. Nutrition*, 1934, **7**, 691.

⁶ Sherman, C. C., Mendel, L. B., and Smith, A. H., *J. Biol. Chem.*, 1936, **113**, 247, 265.

⁷ Langecker, H., *Biochem. Z.*, 1934, **273**, 43.

⁸ Fürth, O. von, Minnibeck, H., and Edel, A., *Ibid.*, 1934, **269**, 379.

⁹ Woods, E., *Am. J. Physiol.*, 1927, **79**, 321.

¹⁰ Hubbel, R. B., Mendel, L. B., and Wakeman, A. J., *J. Nutrition*, 1937, **14**, 273.

inch mesh screen which separated the feces from the urine, which was collected in a flask containing about 20 cc of 5% sulfuric acid. The funnels were rinsed down daily with distilled water. Citric acid was determined by the method of Pucher, Sherman and Vickery,¹¹ the final measurement being made in a photometer using a filter with maximum transmission at 4250 Å.

After an adjustment period of 4 days, urine samples were collected for four 2-day intervals followed by 3 similar periods during which the rats received by stomach tube 400 mg per day of citric acid dissolved in distilled water to make 2 cc. Three more control samples were then collected. The urine samples were diluted to 200 cc and 2 cc aliquots taken for analysis. Later another group of animals was treated by the same procedure except that citric acid determinations were run on both the urine and the feces.

In order to show that the citric acid administered was actually absorbed and not destroyed by the bacteria in the gut, the intestinal contents of two rats were removed. One lot was mixed with citric acid and divided into 4 samples, 2 of which were analyzed immediately for citric acid and the other 2 incubated at 37° for 44 hours in glass stoppered flasks to prevent evaporation. The pH of the other sample of intestinal contents was measured, citric acid added and the pH brought back to the same point by addition of NaOH. This second sample was then treated the same as the first, divided into 4 parts, 2 analyzed immediately and 2 incubated at 37° for 44 hours. The citric acid found in the unbuffered control and incubated samples was 64.7 and 62.7 mg per g respectively and in the buffered samples 62.4 and 62.5 mg per g, showing that the intestinal contents of the rat do not destroy citric acid either at the normal pH of 6.5 or at the pH of 3.3 obtaining after the addition of citric acid.

Urinary pH was measured with a glass electrode on several animals before and after citric acid feeding, the urines being collected under toluene in large test tubes. Only samples uncontaminated with food or feces were used. The values differed by no more than 0.1 pH.

In Table I, the first line for each rat is the average intake and excretion of citric acid for three 2-day control periods immediately preceding the experimental periods. During these control periods each rat was excreting more citric acid in the urine than it was absorbing from the gut and consequently must have been synthesizing citric acid (Smith and Meyer¹²). The following 3 lines for

¹¹ Pucher, G. W., Sherman, C. C., and Vickery, H. B., *J. Biol. Chem.*, 1936, **113**, 235.

¹² Smith, A. H., and Meyer, C. E., *J. Biol. Chem.*, 1939, **131**, 45.

TABLE I.
Disposition of Citric Acid by the Rat.

Rat	Wt	Citric acid intake Mg per 2 days			Citric acid excretion Mg per 2 days			C.A. Abs. %	Excretion of citric acid in % of total intake		
		Food	Extra	Total	Urine	Feces	Total		Urine	Feces	Total
8	256	17.7	0	17.7	25.1	0.84	25.9	95.5	148	4.97	153
		16.3	800	816	20.6	1.03	21.6	99.9	2.46	.123	2.58
		12.9	600	613	31.8	0.56	32.4	99.8	5.19	.091	5.28
		12.2	800	812	39.0	0.62	39.6	99.8	4.81	.076	4.88
9	232	17.0	0	17.0	22.3	1.28	23.6	92.4	142	8.15	150
		15.0	800	815	30.3	1.13	31.4	99.9	3.72	.139	3.86
		12.2	800	812	32.8	0.95	33.8	99.9	4.05	.117	4.17
		12.2	800	812	38.0	0.52	38.5	99.9	4.68	.064	4.74
10	196	17.0	0	17.0	28.7	1.31	30.0	92.4	183	8.34	191
		13.6	600	614	19.1	1.20	20.3	99.8	3.11	.196	3.30
		5.5	800	805	12.2	0.63	12.8	99.8	1.52	.078	1.60
		5.5	800	805	13.6	0.56	14.2	99.9	1.69	.070	1.76
11	156	10.2	0	10.2	19.3	0.43	19.7	96.1	197	4.39	201
		4.8	800	805	15.7	3.49	19.2	99.6	1.96	.435	2.39
		5.5	800	805	18.2	12.35	30.6	98.5	2.29	1.56	3.85
		6.8	400	407	24.2	0.23	24.4	99.9	5.94	.056	6.00
12	290	20.4	0	20.4	74.7	1.72	76.4	91.6	399	9.20	408
		17.7	400	418	60.1	1.10	61.2	99.7	14.4	.264	14.7
		12.9	800	813	52.3	0.97	53.3	99.9	6.44	.119	6.56
		12.9	800	813	72.1	1.72	73.8	99.8	8.86	.212	9.08

each rat give the figures for individual periods during which the rats were receiving extra citric acid. During these periods, with one exception (Rat 11, 3rd period, when he had diarrhea) the absorption of the administered citric acid exceeded 99%, and the percentage of the absorbed citric acid which was excreted in the urine dropped to a very low value. In all 4 of the animals in which only urinary citric acid was followed, there was actually a decrease in the absolute amount of citric acid excreted following the feeding of free acid, and rats 10 and 12 in the table show the same result. In all of the animals, feeding of extra citric acid brought about a remarkable decrease in the percentage of the absorbed citric acid appearing in the urine, showing that under the experimental conditions imposed, the rat possesses the ability to metabolize free citric acid almost completely.

Summary. Citric acid administered to the rat is absorbed since no extra citric acid appears in the feces and intestinal contents do not destroy it. The albino rat has the ability to completely metabolize maximum non-fatal quantities of free citric acid.

11410 P

Occurrence of Special Cell Groups at Vascular Pole of Glomerulus in Mammalian Kidneys.

WILLIAM KAUFMANN. (Introduced by Arthur W. Wright.)

From the Department of Pathology and Bacteriology, Albany Medical College, and the Laboratories of the Albany Hospital, Albany, N. Y.

During the past year we have studied the juxtaglomerular corpuscles of Goormaghtigh¹⁻⁴ in normal and diseased human kidneys removed surgically and at autopsy. This material amounts at the present time to about 200 unselected cases. While similar structures were noted previously by others in laboratory animals,⁵ other mammals⁶ and selected human cases,^{7, 1} this is the first attempt to demonstrate agglomerations of peculiar cells or cell groups around the afferent arteriole of the glomerulus in routine autopsy and surgical material, stained by special as well as routine methods.

These corpuscles are composed of agglomerations of cells, which are situated chiefly at the vascular pole of the glomerulus between the macula densa⁶ of the distal convoluted tubule and the afferent arteriole. A thin layer of these cells may also surround the entire vessel. Occasionally the cells extend along the first part of the arteriole as it enters the glomerular tuft. They usually occur outside the media, surrounding it like a sheath, but they may appear to compose the entire arteriolar wall. We have not observed the corpuscles in kidneys of stillborn infants or children up to 2 years of age. In certain diseased kidneys, as of arteriolar nephrosclerosis, benign or malignant, they may be hypertrophied and thus appear more conspicuous.

The cells which make up the corpuscles can be identified by their morphological characteristics as well as by their staining reactions. They are rather large, polygonal cells with indistinct cell outlines. They are frequently closely packed and delicate argyrophilic fibrils can often be detected between them. Masson's trichrome stain brings out peculiar, fine, fuchsinophilic granules in the cytoplasm, which is

¹ Goormaghtigh, N., *Arch. Biol.*, 1932, **43**, 575.

² Goormaghtigh, N., *J. Physiol.* 1937, **90**, 1263.

³ Goormaghtigh, N., *C. rend. soc. biol.*, 1936, **124**, 293.

⁴ Goormaghtigh, N., and Handovsky, H., *Arch. Path.*, 1938, **26**, 1144.

⁵ Ruyter, J. H. C., *Z. f. Zellforschung*, 1925, **2**, 242.

⁶ Zimmermann, K. W., *Z. f. mikr. anat. Forsch.*, 1933, **32**, 176.

⁷ Oberling, Ch., *Ctes. rend. hebdom. Ac. Sciences, Paris*, 1927, **184**, 1200.

otherwise clear, but vacuolated. The nucleus is large, rounded and vesicular. A clear halo is often seen surrounding it.

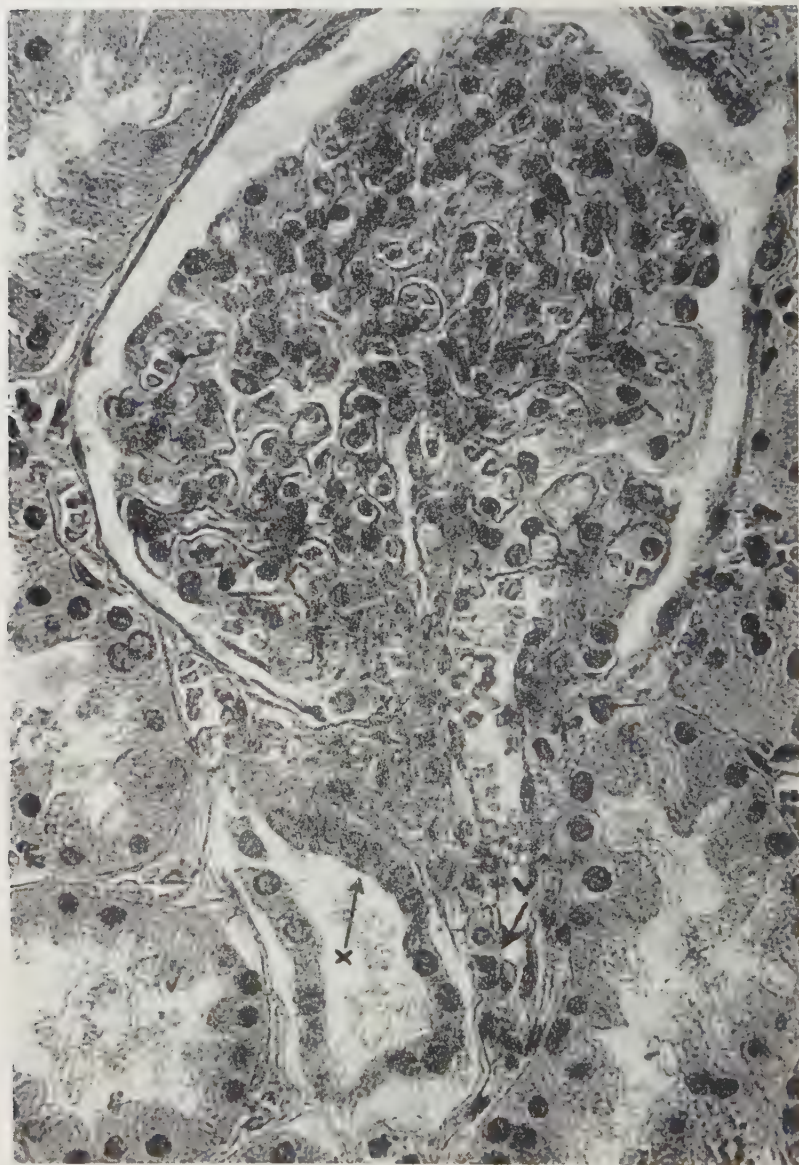


FIG. 1.

Photomicrograph showing a glomerulus of a human kidney with afferent arteriole, juxtaglomerular corpuscle (xx) and adjacent macula densa of the distal convoluted tubule (x). Enlargement 360 \times .



FIG. 2.

Photomicrograph of macula densa (x) and juxtaglomerular corpuscle (xx) in a human kidney. Note the halo around some of the nuclei in the corpuscle and the vacuolization of the cytoplasm. Several intercellular fibrils are also visible. (v) indicates smooth muscle cells of the arteriolar media. Enlargement 600 \times .

Tangential sections of mouse, rat, rabbit and cat kidneys, starting from the cortical surface and proceeding toward the medulla show that the juxtaglomerular corpuscles are absent in the superficial layers of the cortex corticis, but increase markedly toward the middle part of the cortex and decrease again toward the cortico-medullary junction. They seem to be intrinsic characteristic structures of the normal, functioning mammalian kidney. The distribution of the macula densa in the kidney parallels that of the juxtaglomerular corpuscles.

It is important that the tissues be fixed while very fresh, preferably in Bouin's or Zenker's solution. They may be embedded in paraffin in the usual way and sectioned serially, if possible at 4 to 6 microns. Preparations stained with hematoxylin-eosin show the structures clearly, but do not give adequate cytologic details. Masson's trichrome stain is more satisfactory and Mallory's phosphotungstic acid-hematoxylin reveals good nuclear detail. Cytoplasmic vacuolization is clearly visible with this stain and with Mallory's anilin blue connective tissue stain. Intercellular fibrils are well brought out with Masson's stain or better with silver stains.

No definite suggestion as to the nature, the biological or physiological function of these cells can yet be given. Their intimate relationship with the macula densa is noteworthy and may suggest a physio-biologic interrelation between these two structures.

11411

Influence of Neoprontosil on Migration of Blood Leucocytes in Tissue Cultures.*

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Sulfanilamide and Neoprontosil have been reported to stimulate phagocytosis of bacteria by leucocytes *in vitro*. Finkelstein and Birkeland¹ found that in the presence of sulfanilamide and Prontosil (Neoprontosil?) the number of guinea pig leucocytes taking up bacteria and the number of bacteria engulfed per leucocyte was markedly increased. Fresh plasma or serum appeared to be neces-

* The Neoprontosil was furnished by the Department of Medical Research, Winthrop Chemical Co., Inc.

¹ Finkelstein, R., and Birkeland, J. Y., *Science*, 1938, **87**, 441.

sary in order to obtain greater phagocytic activity in the presence of the drugs.

Gay, *et al.*,² however, found no definite difference in the *in vitro* phagocytosis of streptococci by exudate cells from sulfanilamide-treated rabbits as compared with normal rabbits. Streptococci that were treated with sulfanilamide were more readily phagocytized than non-treated streptococci.

Tunnicliff³ observed that both sulfanilamide and Neoprontosil increased the phagocytosis of streptococci by blood leucocytes. She states that "By comparing the amount of phagocytosis in the leukocytes suspended in salt solution with that in leukocytes suspended in dilute prontosil-soluble the stimulating action of prontosil-soluble was observed to be on the leukocytes."

When Neoprontosil in 1:1000 concentration was added to whole blood with bacteria, the number of cocci ingested per leucocyte was doubled. Serum from mice receiving molar equivalents of sulfanilamide and Neoprontosil increased the phagocytic activity of normal leucocytes to the same degree.

King⁴ demonstrated that sulfanilamide 1:1000 stimulated the rate of migration of rabbit leucocytes in sterile tissue cultures. After 24 hours of incubation the average migration rims were 18% wider than in the controls. The absolute difference was 5.8 times the standard error of the difference.

The influence of Neoprontosil (the disodium salt of 4' sulfamido-phenyl-2-azo-7 acetylamino-1 hydroxynaphthalene-3, 6 disulfonic acid) on the migration rate of blood leucocytes in sterile tissue cultures was studied.

The routine culture methods used were described by King.^{5, 6} To obtain the buffy coat, 15 cc of rabbit blood were drawn into a 50 cc centrifuge tube containing sufficient heparin to prevent clotting. The blood was centrifuged at high speed for 15 minutes and the plasma removed. The tube was recorked with a fresh sterile cork and re-centrifuged for one-half hour. If the tubes are handled carefully, the buffy coat can at the end of the second centrifugation be removed as a solid plaque. After washing the buffy coat with Tyrode to remove the adhering red cells, it was fragmented and covered with Tyrode. Buffy coat fragments are extremely fragile. If allowed to

² Gay, F. P., Clark, A. R., Street, J. A., and Miles, D. W., *J. Exp. Med.*, 1939, **69**, 607.

³ Tunnicliff, R., *J. Inf. Disease*, 1939, **64**, 59.

⁴ King, J. T., *Am. J. Physiol.*, 1938, **123**, 119.

⁵ King, J. T., *Arch. f. Exp. Zellforsch.*, 1930, **9**, 341.

⁶ King, J. T., *Arch. f. Exp. Zellforsch.*, 1931, **10**, 467.

remain in a fluid medium for more than one hour or handled roughly, they disintegrate rapidly.

The fragments, 2 to 3 mm in diameter, were carefully chosen in pairs according to size, shape and general texture. One fragment from each pair was used as the control, the other fragment as the experimental tissue. In this way each control culture has a visually identical experimental culture. Each series contained 30 cultures made from one animal.

The cultures were planted in moist chambers (Maximow technic) and incubated as lying drops in a special down-draft incubator described by King.⁷

The fragments were planted in one part autogenous heparinized plasma and 3 parts of an autogenous rabbit serum extract of 6-day chick embryos. Sufficient Neoprontosil was added to the serum extract to make a 1:1000 concentration in the final culture medium.

Observations were made on the living cultures at the end of 24 hours of incubation. The maximum migration rim was measured with a 16 mm objective and a 6× ocular with an eyepiece micrometer (114 units = 1 mm).

TABLE I.

No. of series	No. of cultures per series	Avg control	Avg experimental	Absolute difference	S.E. of difference	% increase
16	30	220	242.8	22.8	7.48	10.3

The experimental data are shown in Table I. From the results it appears that Neoprontosil 1:1000 increases the rate at which rabbit blood leucocytes migrate in tissue culture media. The absolute difference in the width of the migration rims between the experimental and control cultures is 22.8 and the standard error of the difference is 7.48.

Whether there is any significant correlation between the increased migration and the increased phagocytosis by leucocytes *in vitro* is not clear at present.

The possibility that the Neoprontosil might be partially split into sulfanilamide in tissue culture media and exert its stimulating effect as such must be considered. No experimental evidence is available on this point.

Conclusion. In tissue culture media composed entirely of animal body fluids Neoprontosil 1:1000 stimulates the rate of rabbit blood leucocyte migration.

⁷ King, J. T., *Arch. f. Exp. Zellforsch.*, 1937, **20**, 208.

11412 P

Experimental Coronary Occlusion and Myocardial Fibrosis.

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Detroit, Mich.*

Colloidal solution of aluminum hydroxide was observed by Beland, Moe, and Visscher¹ to produce rapid stoppage of the heart when the aluminum was perfused into the coronary arteries in a heart-lung preparation. They believed that coronary insufficiency resulted from multiple capillary emboli due to the injection of the aluminum hydroxide. A 2% aluminum hydroxide solution was also injected into the right side of the heart in a similar preparation. In this instance the lungs apparently filtered out sufficient aluminum so that no deleterious effect was noted on the coronary circulation. Irwin² studied the effect of aluminum solutions injected intravenously in experimental animals. He found that if sufficient quantities of the metal were injected the animal died due to pulmonary emboli. Nodular fibrotic areas were noted in the lungs of rabbits following experimental inhalation of aluminum dust;³ the tissue response was that of a foreign body reaction.

In the present study the sternums of 4 control rabbits were removed so that aluminum hydroxide could be injected directly into the cavity of the beating left ventricle. Large doses of the material were injected until the animals died. No gross changes were apparent in the heart or other organs. Microscopic study of various sections of the heart muscle with the hematoxylin and eosin stain reveals numerous small arterioles to be filled with bluish-purple masses which partially or completely occluded the smaller arterioles. In those vessels only partially occluded with the colloidal material the aluminum was found to adhere to the wall of the vessel.

In order to prove that the suspected masses were aluminum the sections were stained for aluminum using a specific stain. The aluminum particles were noted to be cherry-red in color by this method. In some instances the very small aggregates of the material seen in small capillaries with the hematoxylin and eosin stain failed to take the red color with a specific stain (Aurine). This

¹ Beland, I. J., Moe, G., and Visscher, M. D., *PROC. SOC. EXP. BIO. AND MED.*, 1938, **39**, 145.

² Personal communication.

³ Denny, J. J., Robson, M. B., and Irwin, D. A., *Canad. Med. Assn. J.*, 1937, **37**, 1.

may be due to the small quantities of the aluminum present. After some experience the small aluminum thrombi can be detected with little difficulty, using hematoxylin and eosin stain following formalin fixation.

Thirty rabbits were given repeated intraventricular injections of aluminum hydroxide in non-fatal amounts. In animals weighing from 1800 to 2500 g, 1 cc of the 2% solution could be safely given. Twenty-two of the rabbits lived and were sacrificed from one to 6 weeks following repeated injections of the aluminum into the left ventricle while others died immediately following such injections.

Gross examination of the hearts of the 22 animals which survived revealed fibrotic patches in some. A few yellowish areas measuring one millimeter in diameter could be observed in others. Microscopic examination of the heart muscle of these animals revealed numerous small fibrotic patches scattered particularly throughout the left ventricle. These scarred areas are similar to those seen in human cases that are associated with coronary sclerosis. In a few of the sections a chronic inflammatory reaction was the most pronounced change present.

A number of the hearts showed small areas of infarction, particularly in the lower third of the septum and near the tip of the left ventricle. Some of the small arterioles seen in the adjacent myocardium were partially or completely occluded by aluminum thrombi. The cardiac muscle adjacent to the infarcts was partially replaced by actively proliferating fibroblasts and numerous inflammatory cells. The latter consisted of lymphocytes, eosinophils and numerous macrophages.

A more chronic proliferative lesion was noted in some areas of the heart in which large amounts of aluminum were present. In addition to the partial occlusion of the vessels some of the material apparently incited a foreign body reaction. The macrophages in those sections contained numerous particles of aluminum. A few giant cells of the foreign body type were also seen in the nearby tissue. The marked proliferative response of the fibroblasts in some instances produced a picture similar to true Aschoff nodules. Changes in the vessels were not especially frequent; the change most commonly observed was some intimal proliferation and an increased thickness of the vessel wall.

Summary. It is possible to produce coronary occlusion and myocardial fibrosis experimentally in the rabbit by means of injection of colloidal aluminum hydroxide into the left ventricle. This method may be useful in the experimental study of cardiac hypertrophy and coronary disease.

11413 P

Liberation of a Histamine-Like Substance on Stimulation of Sympathetic Nerves.

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Several attempts have been made to find a nervous mechanism for the release of histamine from body tissues,^{1, 2} but thus far the only evidence that such may exist has been indirect.³ The present study deals with the release of histamine by the skin upon stimulation of sympathetic nerves. The epidermal layer of the skin contains relatively large quantities of histamine (24 γ /g).

Experiments were carried out on rabbits, unanesthetized or anesthetized lightly with urethane or ether. The cervical sympathetic trunk and its superior ganglion were prepared for stimulation with bipolar electrodes (4 to 60 sec, primary 3 volts, secondary coil at 10 cm). Blood samples obtained from the great auricular vein by venipuncture before and after nerve stimulation were compared for their ability to contract an isolated segment of guinea pig ileum according to the method of Schultz and Dale. Atropinized Ringer-Locke solution was used in the muscle bath in every case. Standard histamine solutions were used for comparison of contractions.

In early experiments the blood samples were allowed to clot; the serum obtained was diluted immediately with Ringer's solution or buffer solution of pH 7.15 and tested on the guinea pig ileum. In these experiments (10 animals) serum obtained 15 to 60 seconds following nerve stimulation almost invariably produced a greater contraction of the guinea pig ileum than the control serum (15 to 50% greater). This difference was present after and often increased by heating the diluted sera in a water bath at 60° to 70°C for 30 minutes, provided the pH of the sera was not above 7.15. The entire contractor effect of both control and stimulation sera could be abolished by previous addition of 0.5 γ of thymoxyethyldiethylamine to the muscle bath.

Rabbit blood has already a high content of histamine which is almost entirely stored in the cellular elements (platelets, Minard⁴),

* Partially aided by a grant from Abbott Laboratories.

¹ MacGregor, R. R., and Peat, S., *J. Physiol.*, 1931, **71**, 31.

² Bulbring, E., and Burn, J. H., *J. Physiol.*, 1935, **83**, 483.

³ Lewis, T., and Marvin, H. M., *Heart*, 1927, **14**, 27.

⁴ Minard, D., *Am. J. Physiol.*, 1937, **119**, 375.

but is liberated into the serum after clotting.⁵ Since only that histamine found free in the blood plasma is active *in vivo*, an attempt was made to determine whether there was an increase in plasma histamine after nerve stimulation (5 animals). Whole blood (0.1 to 0.2 cc) was drawn directly into a syringe containing an equal quantity of heparinized Ringer's solution. These were drawn, mixed, and added at once to the muscle bath. It was found that control samples of unclotted blood contained no demonstrable contractor substance. However, following nerve stimulation, ability to contract the muscle appeared in $\frac{1}{2}$ to 3 minutes and was usually undetectable again after 8 to 15 minutes. This property of the whole blood could be abolished by previous addition of thymoxyethyl-diethylamine to the muscle bath. The experiment could be repeated more than once using the same rabbit ear.

Two experiments with cats, testing blood serum, gave results similar to those in the rabbit. Blood was drawn from the external jugular vein following stimulation of the superior cervical sympathetic ganglion.

Adrenalin which inhibits to some extent the muscle contraction produced by histamine is liberated on stimulation of sympathetic nerves to the rabbit's ear.⁶ Frequently, in our own experiments, blood drawn immediately following stimulation inhibited the effect of standard histamine solutions added to the bath more than did control samples. Our results may have been made less apparent by this antagonism.

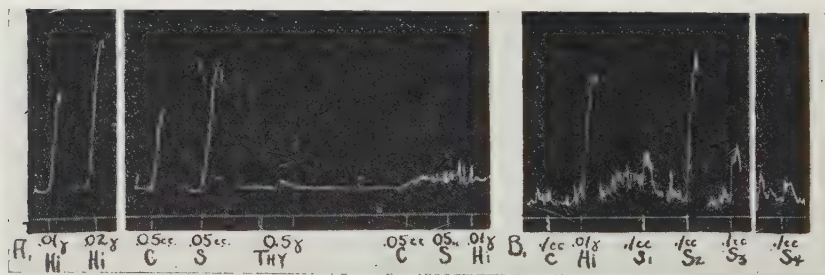


FIG. 1.

A. Effect of thymoxyethyl-diethylamine on C, control serum, on S, serum obtained after stimulation of the superior ganglion (4 sec), and on Hi, standard histamine.

B. Effect on the ileum of C, control heparinized, whole blood; S₁, obtained immediately after stimulation of ganglion (10 sec); S₂, 2 min after stimulation; S₃, 4 min after stimulation; S₄, 13 min after stimulation.

⁵ Code, C. F., *J. Physiol.*, 1937, **90**, 349.

⁶ Gaddum, J. H., Jang, C. S., and Kwiatkowski, H., *J. Physiol.*, 1939, **96**, 104.

The identification of the contractor substance is aided by the use of thymoxyethyldiethylamine. This drug is specific in counteracting the effect of histamine on the guinea pig ileum.⁷ It has no comparable effect upon contractions produced by KCl, NaHCO₃, acetylcholine, or the contraction produced in rare instances by adrenaline. Acetylcholine produces no contraction of an atropinized muscle. The slight changes in pH of the blood following nerve stimulation were not sufficient to affect the activity of the muscle strip and most sera were diluted with a buffer solution. On the other hand, the contractor substance obtained in the blood from the rabbit's ear following nerve stimulation was heat stable, active in an atropinized bath, but inactive after addition of thymoxyethyldiethylamine. In these respects it is "histamine-like". The source of this substance in the rabbit's ear has not been determined.

We wish to acknowledge the assistance of Mr. C. J. Loechl in these studies.

11414 P

Thymoxyethyldiethylamine Antagonism to Circulatory Effects of Histamine in Anesthetized and Nonanesthetized Dogs.

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Although thymoxyethyldiethylamine (Thym.) has been found to antagonize histamine (Hi) effects on isolated smooth muscle¹⁻⁴ and to exert a protective action in guinea pigs against anaphylactic and Hi shock,^{2, 3, 5} little work has been done regarding its action in carnivorous animals in which the circulatory effects of Hi are most striking. We have undertaken to investigate Thym. antagonism to the hypotension and hemoconcentration resulting from Hi administration in anesthetized and nonanesthetized dogs, and to observe the effects of Thym. alone.

⁷ Rosenthal, S. R., and Minard, D., *J. Exp. Med.*, 1939, **70**, 415.

¹ Bovet, D., and Staub, A. M., *C. R. Soc. Biol.*, 1937, **124**, 547.

² Staub, A., and Bovet, D., *C. R. Soc. Biol.*, 1937, **125**, 818.

³ Staub, A. M., *Ann. Inst. Pasteur*, 1939, **63**, 400, 485.

⁴ Rosenthal, S. R., and Minard, D., *J. Exp. Med.*, 1939, **70**, 415.

⁵ Rosenthal, S. R., and Brown, M. L., *J. Immunol.*, in press.

In the first series of experiments, dogs under light amytal anesthesia (60 mg/kg intraperitoneally) were used. Mercury manometer recording of carotid pressure, tracheal cannulation with pneumometer recording of respiration, and cannulation of the femoral vein for injection completed the preparation.

Four dogs served to establish the effects of small (1-10 γ) and large (.25-1.0 mg/kg) doses of Hi injected intravenously. Accompanying the blood pressure fall after large Hi doses, signs of severe respiratory obstruction were observed and taken to indicate bronchial constriction.

In 10 dogs prepared as above, depressor effects of 1-10 γ doses of Hi were studied before and after subcutaneous administration of 40 mg/kg Thym. After Thym. the depressor effects of these Hi doses were markedly decreased. Acetylcholine responses were affected but slightly, thus indicating a specificity of Thym. action similar to that observed on isolated smooth muscle.⁴

TABLE I.
Depressor Responses to Hi and Acetylcholine Before and After Thym.

	Before Thym.	After Thym.
Hi 4 γ	39 mm Hg	10 mm Hg
Ac 2 γ	40	30

After large Hi doses, the drug had no apparent effect either on the degree of blood pressure fall or recovery rate; however, indications of bronchial constriction were no longer evident.

In the second series of experiments, using nonanesthetized dogs, arterial pressure readings from the femoral artery exposed under local anesthesia were obtained with a needle and anaeroid manometer, a spinal fluid trap being interposed. Blood samples were drawn and injections were made into the femoral vein. Hemoglobin concentrations were determined by the Sanford-Sheard photelometer.⁶

The effects of subcutaneous administration of 40 mg/kg Thym. on pulse rate, blood pressure, and hemoconcentration were observed in 5 animals. The pulse and blood pressure uniformly showed a rise in 5-10 minutes after injection, reached a peak in 30-45 minutes, and gradually declined to normal. The hemoglobin level usually showed a transitory rise of less than 10%.

The effects of Hi (1 mg/kg intravenously) were studied in 16 experiments on 9 dogs with and without previous Thym. administration. The results, summarized in Table II, indicate a significant

⁶ Sanford, A. H., Sheard, C., and Osterberg, A. E., *Am. J. Clin. Path.*, 1933, **3**, 405.

TABLE II.
Hemoconcentration After Hi in Nonanesthetized Dogs With and Without Thym.
Treatment.

No. of expts.	Maximum hemoconc., avg (% of initial level)		Recovery time, avg (To 10% of initial level)	
	Histamine	Thym. + Histamine	Histamine	Thym. + Histamine
9	26.5			
7		11.8	> 110 min	30 min

reduction in both the degree and duration of hemoconcentration following histamine in animals receiving Thym. On the other hand, the blood pressure effects of Hi (1 mg/kg) were not appreciably altered by Thym. treatment.

Discussion. Staub³ failed to observe a decrease in the depressor response to Hi in chloralosed dogs after intravenous Thym. injection, probably because the Hi doses used (.02 mg/kg) were beyond the effective range of Thym. However, we can confirm in dogs this author's observations in guinea pigs on Thym. antagonism to bronchoconstrictor effects of Hi.

Insufficient experimental work precludes any postulation regarding the mechanism of Thym. antagonism to the depressor effects of small Hi doses. However, it seems unlikely that Hi vasodilatation is involved; more probably Thym. action is limited to abolishing venoconstrictor effects of Hi.

Whether the marked hemoconcentration observed in nonanesthetized dogs after Hi represents solely a loss of plasma volume or whether an influx of red cells from a reservoir such as the spleen is an important factor has not been investigated. Hence, it is premature to suggest that Thym. may reduce loss of plasma volume in histamine shock.

Summary. Thymoxyethyl-diethylamine administered subcutaneously to (a) anesthetized and (b) nonanesthetized dogs reduces or abolishes the depressor effects of small histamine (Hi) doses and prevents the bronchial constrictor action of large Hi doses in (a) and reduces the hemoconcentration following large Hi doses in (b), but in neither (a) nor (b) are the depressor effects of large Hi doses appreciably altered.

Zinc Salts and Oil in Prolongation of Therapeutic Effect of Pitressin in Experimental Diabetes Insipidus.

D. J. STEPHENS. (Introduced by W. S. McCann.)

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It has been shown by previous investigators that the addition of zinc salts increases the activity of hypophyseal gonadotropic extracts^{1,2} and prolongs the hypoglycemic effect of insulin.³ Boyd and Clark⁴ have reported that the addition of zinc salts to posterior pituitary extracts prolongs the retention of water taken up by frogs. Keeney, Pierce and Gay⁵ have shown that the effect of epinephrine is greatly prolonged when it is given suspended in oil. The present communication is a preliminary report of an investigation of the effect of zinc salts and of oil on the action of pitressin in experimental diabetes insipidus.

A dog with experimental diabetes insipidus⁶ was loaned to the author for this study by Dr. Roland Bellows of the Department of Neurosurgery. The animal was fed once daily at 9 A.M.; liberal amounts of drinking water were available at all times. The urine was collected in fractional samples every 4 hours, with the exception of the period between 1 A.M. and 9 A.M., when a single 8-hour specimen was collected. The urine volume and specific gravity were accurately measured.

Test substances were injected in a single dose at 9 A.M. The results of the subcutaneous injection of 1 cc of 0.2% zinc acetate solution, of 1 cc of pitressin* and of a mixture of 1 cc of pitressin and 1 cc of 0.2% zinc acetate solution are shown in Table I. The subcutaneous administration of zinc acetate alone had no effect. The administration of a single dose of 20 units of pitressin resulted in a moderate reduction of the urine volume. The pitressin effect was

¹ Fevold, H. L., Hisaw, F. L., and Greep, R., *Am. J. Physiol.*, 1936, **117**, 68.

² Saunders, F. J., and Cole, H. H., *Proc. Soc. Exp. Biol. and Med.*, 1936, **33**, 505.

³ Scott, D. A., and Fisher, A. M., *J. Pharm. and Exp. Therap.*, 1935, **55**, 206.

⁴ Boyd, E. M., and Clark, K. J., *Am. J. Med. Sci.*, 1939, **198**, 171.

⁵ Keeney, E. L., Pierce, J. A., and Gay, L. N., *Arch. Int. Med.*, 1939, **63**, 119.

⁶ Bellows, R. T., and VanWagenen, W. P., *J. Nerv. and Mental Dis.*, 1938, **88**, 417.

* The pitressin and the pitressin-in-oil used in this study were generously supplied by Dr. E. A. Sharp of the Parke-Davis Co.

TABLE I.

Treatment	No. of Exp.	Avg urine volume in cc					24 hr Total
		9-1	1-5	5-9	9-1	1-9	
Control	15	980	1410	950	660	975	4975
1 cc 0.2% ZnAc	4	1110	1230	870	650	1120	4980
1 " pitressin	7	290	380	500	540	980	2690
1 " 0.2% ZnAc	8	260	150	190	140	690	1430
1 " pitressin							
Pitressin-in-oil	3						
Control day		970	1250	970	640	1100	4930
First " "		445	320	50	120	200	1135
Second " "		0	320	220	80	490	1110
Third " "		200	770	870	290	780	2910
Fourth " "		860	1030	510	350	1000	3750
Fifth " "		700	1550	1160	460	980	4850

*Pitressin-in-oil, 1.3 cc (20 units), injected intramuscularly at 9 A.M., on the first day only.

most striking during the first 4 hours, with some reduction in urine volume for as long as 12 hours after injection. The administration of a mixture of 1.0 cc of pitressin and 1.0 cc of 0.2% zinc acetate solution resulted in prolongation and intensification of the pitressin effect during the period from 4 to 16 hours after injection. The effect of both the aqueous pitressin and the zinc-pitressin mixture was dissipated within 24 hours.

The effect of the administration of a preparation of pitressin suspended in peanut oil was more striking. The results of 3 experiments are summarized in Table I. A single intramuscular injection of 1.3 cc (20 units) of pitressin-in-oil resulted in a maximal pitressin effect of fully 48 hours' duration, as contrasted with a 4- to 8-hour maximum effect in the case of a similar dose in aqueous solution and a 16-hour maximum effect when given in an aqueous solution of a mixture of pitressin and zinc acetate. With the oil preparations there was some effect evident for as long as 72 hours.

These observations suggest that when pitressin is given alone, in watery solutions, it is rapidly absorbed and its full effect lost through destruction or excretion of the active principle. The data presented in this communication are compatible with the view that absorption of the pitressin is delayed when given in the presence of zinc acetate, or in oil.

Summary. The presence of 0.1% zinc acetate prolonged and intensified the effect of aqueous solutions of pitressin in reducing the water exchange in experimental diabetes insipidus. The use of a preparation of pitressin suspended in oil resulted in a much more marked prolongation and intensification of the pitressin effect.

Comparative Curative Values of Unsaturated Fatty Acids in Fat Deficiency.*

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Some time ago¹ we called attention to the fact that cod liver oil fed at 2-5 drop levels daily caused renewed growth of fat-deficient rats but did not clear the scaly skin. In recent years we have had occasion to test numerous oils and pure fatty acids for these effects. Some of the results are reported in this paper.

Fat deficiency in white rats was produced with the diet 550-B used by Burr and Burr² consisting of casein, sucrose and salts supplemented with whole dried yeast (Northwestern) and the unsaponifiable fractions of cod liver oil and wheat germ oil. When a growth plateau is reached and the skin has become scaly the animals are used for a study of the curative effects of the oils and fatty acids. The results are recorded as gains in weight in a given time and in apparent change in skin condition. An arbitrary rating of scale on the feet, scale on the tail and dandruff in the hair, ranging from 0 to 3 (most severe) is summed to give a maximum scaly condition of 9. Three to 6 rats are used in each group and the results have been repeated several times.

During a search of corn oil, linseed oil and codliver oil for isomeric fatty acids the oils were fed at low levels and the responses noted at the end of 4 weeks. The results are recorded in Fig. 1. Ten mg daily of corn or linseed oil is not sufficient to produce marked improvement. At the 20 and 40 mg levels, however, all 3 oils give good growth responses. On the other hand, corn oil is the only one which produces a complete skin cure in this period of time. These differences can be explained if we assume that linoleic acid is responsible for the clearing of the skin, while linolenic and cod liver oil acids have little or no such effect. Corn oil (Mazola) has over 60% linoleic acid. Linseed oil probably has 30% linoleic and 40% linolenic acids.

* Assistance in the preparation of these materials was furnished by the personnel of Works Projects Administration, Official Project No. 65-1-71-140, Subproject No. 325.

¹ Burr, G. O., Burr, M. M., and Brown, W. R., *PROC. SOC. EXP. BIOL. AND MED.*, 1931, **28**, 905.

² Burr, G. O., and Burr, M. M., *J. Biol. Chem.*, 1929, **82**, 345.

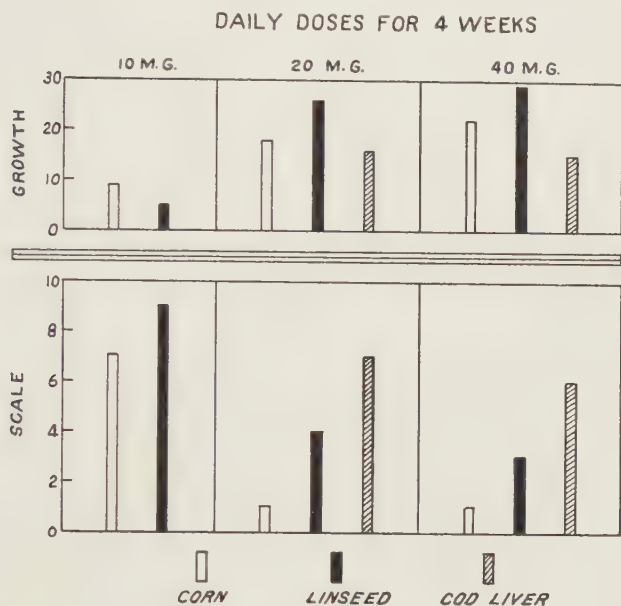


FIG. 1.

Growth and skin cure during 4 weeks of feeding oils at 3 levels. Poor skin cure is indicated by high column for scale.

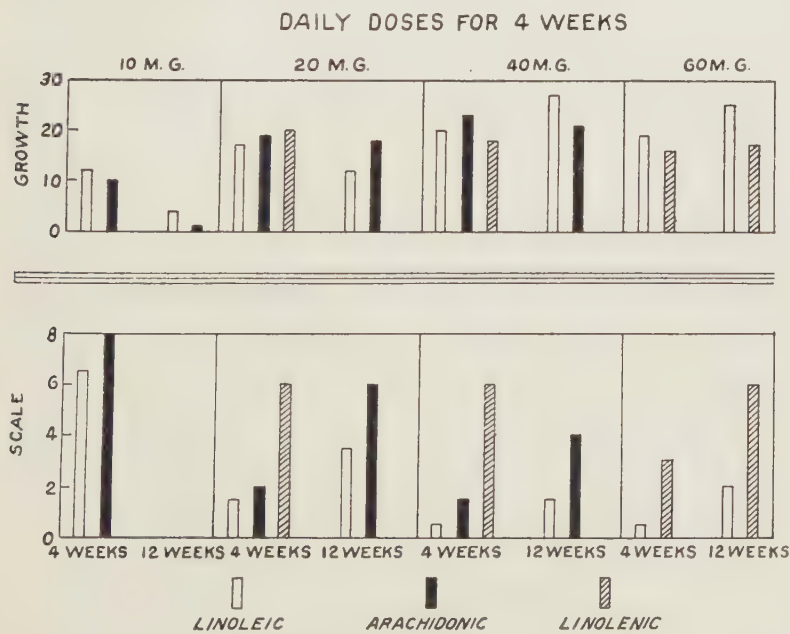


FIG. 2.

Growth and skin cures with 3 pure fatty acids. The doses were fed daily for 4 weeks and stopped, while growth and skin records were continued 8 more weeks to show storage effects.

In Fig. 2 are summarized the results of feeding linoleic, linolenic and arachidonic acid. In these experiments the doses were fed for only 4 weeks and the observations continued for 8 more weeks to measure the storage effects. It is clear that linolenic acid gives good growth responses but has little effect on the skin. Arachidonic and linoleic acids are similar in their effects. When fed at these low levels we do not find the superior action of arachidonic acid cited by Turpeinen.³ When fed at a 40 mg level there is a storage effect of linoleic acid which makes those animals superior in both weight and skin quality after 8 weeks on the fat-deficient ration.

These findings are of particular interest at this time in the study of the relation of unsaturated acids to vitamin B₆ deficiency. It has been observed by numerous workers that certain oils cure the severe acrodynia accompanying B₆ deficiency. Schneider, Ascham, Platz and Steenbock⁴ recently summarized their findings on the anti-acrodynic potency of foods. Examination of their Table I shows that cod liver oil is very poor and the linolenic acid of linseed oil is ineffective. Those oils highest in linoleic acid are most effective (corn oil and wheat germ oil). Salmon⁵ recently reported that methyl linolate was much more effective than methyl linolenate in curing the skin of B₆ deficient rats.

Conclusions. Unsaturated fatty acids (linoleic, linolenic, arachidonic and cod liver oil acids) show differences in growth and skin effects. They should no longer be treated as an interchangeable group but should be used individually in nutrition studies.

³ Turpeinen, O., *J. Nutr.*, 1938, **15**, 351.

⁴ Schneider, H. A., Ascham, J. K., Platz, B. R., and Steenbock, H., *J. Nutr.*, 1939, **18**, 99.

⁵ Salmon, W. D., *Proc. Soc. Biol. Chem.*, 1940, **31**, 83.

11417

Pyruvic Acid in Blood and Cerebrospinal Fluid.*

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It has been shown that cocarboxylase (the diphosphoric ester of vitamin B₁) is necessary for the normal catabolism of pyruvic acid.¹ In vitamin B₁ deficiency (Oriental Beri-beri) pyruvic acid accumulates in the body fluids.² Methods previously used in the determination are not satisfactory since a significant decrease in blood pyruvate occurs if the blood be allowed to stand at room temperature for even one minute prior to precipitation. This disappearance does not occur if moniodoacetate in a concentration of 0.2% is used as a stabilizing medium. Utilizing this finding, we have recently described a method for the stabilization and determination of pyruvic acid in the blood.³

The present study was undertaken in order (1) to determine the degree of stability of pyruvic acid in the spinal fluid, (2) to compare the levels of pyruvic acid in the blood and cerebrospinal fluid, (3) to make certain preliminary observations concerning the value of pyruvic acid determinations for the diagnosis of vitamin B₁ deficiency.

Method. The method for blood determinations was also used for the spinal fluid, except that the stabilizing medium (moniodoacetate) was found to be unnecessary.

Stability of Pyruvic Acid in the Spinal Fluid. In contrast to our findings in the blood, the pyruvic acid content of the spinal fluid remains constant even if the sample be allowed to stand at room temperature for one hour prior to precipitation. Additional evidence of the stability of pyruvic acid in the spinal fluid was obtained in the following way: Five cc samples of cerebrospinal fluid were caught in test tubes containing 25 mg of sodium moniodoacetate, and other samples of the same spinal fluid were allowed to stand at room temperature for 60 minutes prior to precipitation without the use of the

* This work was aided by a grant from Child Neurology Research (Friedsam Foundation).

1 Banga, I. L., Ochoa, S., and Peters, R. A., *Biochem. J.*, 1939, **33**, 1109.

2 Platt, B. S., and Lu, G. D., *Quart. J. Med.*, 1936, **5**, 355.

3 Bueding, E., and Wortis, H., *J. Biol. Chem.*, 1940, **133**, 585.

TABLE I.
Stability of Pyruvate in the Spinal Fluid.

Spinal fluid sample No.	Precipitated after 3 min mg% pyruvic acid	25 mg CH_2ICOONa added.	No CH_2ICOONa added.
		Precipitated after 3 min mg% pyruvic acid	Precipitated after 60 min mg% pyruvic acid
1	0.90	—	0.88
2	0.93	—	0.93
3	—	0.86	0.86
4	—	1.04	1.05
5	—	0.83	0.83
6	1.12	1.09	1.10
7	0.78	—	0.79
8*	—	2.22	2.18
9	—	2.40	2.31

*8 and 9 were cases of pneumococcus meningitis.

stabilizing medium. Identical values were obtained in both samples (Table I).

Pyruvic Acid in the Blood and Cerebrospinal Fluid. Simultaneous samples of blood and cerebrospinal fluid were obtained on 67 patients with various neuropsychiatric and medical disorders. The subjects were fasting and at rest in bed. All determinations were done in duplicate.

The content of blood pyruvic acid in 60 normal subjects was previously³ found to vary from 0.77-1.16 mg %. We have, therefore, arbitrarily decided to consider as abnormally high those cases with blood pyruvate levels of 1.30 mg % or above.

Considered in this fashion, 51 of our 67 cases had normal values for pyruvic acid in the blood, and 16 of our cases showed elevated figures. The relationship of spinal fluid pyruvate to that of the blood in these 2 groups is seen in Table II.

Preliminary Observations Concerning the Value of Pyruvic Acid Determinations for the Diagnosis of Vitamin B₁ Deficiency. The 51 cases with normal blood levels formed the following diagnostically labelled groups: (a) chronic alcoholism without clinical evidence of vitamin B₁ deficiency (peripheral neuropathy or beri-beri), 15 cases;

TABLE II.
Relationship of Blood to Spinal Fluid Pyruvate.

Group	No. of cases	Mg% pyruvic acid		
		Blood	C.S.F.	% C.S.F./ blood
1	51	0.79-1.30 (Avg 1.03)	0.42-1.52 (Avg 0.84)	43-118*
2	16	1.41-2.41 (Avg 1.82)	1.03-2.40 (Avg 1.77)	67-170*

* In only 6 cases (2 in Group 1 and 4 in Group 2) did the % C.S.F./Blood fall outside the range 70-120%.

(b) Schizophrenia, 10 cases; (c) behavior problems in children, 6 cases; (d) mental deficiency in children, 5 cases; (e) pneumonia, 5 cases; (f) psychopathic personality, 2 cases; (g) hyperthyroidism, 2 cases; (h) senile psychosis, 2 cases; (i) one case each of arsenical poisoning, reactive depression, paroxysmal convulsive disorder of unknown etiology and herpes zoster.

The 16 cases with high blood levels formed these diagnostically labelled groups: (a) chronic alcoholism with acute peripheral neuropathy, 12 cases; (b) beri-beri, 1 case; (c) pneumococcus meningitis, 2 cases; (d) pneumonia with prolonged temperature elevation, 1 case.

Of the 16 cases with elevated pyruvic acid levels, it is noteworthy that 13 (12 of acute peripheral neuropathy and one of beri-beri) occurred in clinical syndromes which are known to be the result of vitamin B₁ deficiency. As a matter of fact, Platt and Lu have previously described elevations of blood pyruvate in cases of fulminating beri-beri.^{2, 4} We have criticized the limitations of their method in previous communications.^{3, 5} In the 3 remaining cases with elevated blood pyruvate, the increase in total metabolism, as a result of prolonged fever with resultant depletion of vitamin B₁, may have contributed to the high values obtained. It should, however, be particularly noted that none of these cases showed evidences of acute peripheral neuropathy. It may be that the metabolic disturbance must exist for some time before clinical evidences of vitamin B₁ deficiency are apparent. In addition, further work may indicate that elevations in blood pyruvate are related to conditions other than avitaminosis B₁.

Nonetheless, our results take on added interest if we reexamine our cases of chronic alcoholism without clinical evidences of vitamin B₁ deficiency. These 15 cases are subdivided as follows: (a) chronic alcoholism with old or treated peripheral neuropathy, 8 cases; (b) chronic alcoholism without evidences of involvement of the central or peripheral nervous systems, 4 cases; (c) chronic alcoholism with nicotinic acid deficiency, 2 cases; (d) chronic alcoholism with brain laceration, 1 case. The pyruvic acid levels were normal in every case. On the other hand, our cases of chronic alcoholism with clinical evidences of vitamin B₁ deficiency showed an elevation of blood pyruvate in every instance.

Summary. 1. The stability of pyruvic acid in the spinal fluid is

⁴ Lu, G. D., *Biochem. J.*, 1939, **33**, 774.

⁵ Wortis, H., Bueding, E., and Wilson, W., *PROC. SOC. EXP. BIOL. AND MED.*, 1940, **43**, 279.

described. 2. The relationship of blood to spinal fluid pyruvate is reported. The amount found in the cerebrospinal fluid is usually 70-120% of that found in a blood sample taken simultaneously. 3. Of the 16 cases with elevated blood pyruvate, 13 occurred in cases of known vitamin B₁ deficiency. These latter constituted the only cases in the entire study with definite clinical evidences of vitamin B₁ deficiency. In the other 3, it is suggested that a relative deficiency of vitamin B₁ may have existed. In 51 additional cases, without clinical evidence of vitamin B₁ deficiency, the blood pyruvate was normal in every instance.

11418 P

A New Type of Vitamin K-Deficient Diets.

S. ANSBACHER.

From The Squibb Institute for Medical Research, New Brunswick, N. J.

Heretofore the vitamin K-deficient Diet E of Almquist and Stokstad,¹ Ration K-1 of Ansbacher,² Diet 508 of Dam and co-workers,³ or modifications thereof, have been used by the majority of investigators¹⁻⁷ in assays, in which chicks served as test animals. Since putrified fish meal is an excellent source of one of the natural antihemorrhagic vitamins, obstacles are frequently encountered in the employ of fish meal diets even under conditions tending to minimize bacterial action.

Recently we found that the difficulties arising from K-vitamin synthesis are not experienced when diets are used which contain neither fish meal nor yeast, and in which vitamin K had been destroyed by prolonged heat treatment. We are now making vitamin K assays with Ration K-7 outlined in the accompanying table.

¹ Almquist, H. J., and Stokstad, E. L. R., *J. Nutrition*, 1936, **12**, 329.

² Ansbacher, S., *J. Nutrition*, 1939, **17**, 303.

³ Dam, H., Glavind, J., and Karrer, P., *Helv. Chim. Acta.*, 1940, **23**, 224.

⁴ Dann, F. P., *PROC. SOC. EXP. BIOL. AND MED.*, 1939, **42**, 663.

⁵ MacFie, J. M., Bacharach, A. L., and Chance, M. R. A., *Brit. Med. J.*, Dec. 23, 1939, 1220.

⁶ Thayer, S. A., McKee, R. W., Binkley, S. B., MacCorquodale, D. W., and Doisy, E. A., *PROC. SOC. EXP. BIOL. AND MED.*, 1939, **41**, 194.

⁷ Tidrick, R. T., Joyce, F. T., and Smith, H. P., *PROC. SOC. EXP. BIOL. AND MED.*, 1939, **42**, 853.

RATION K-7.			%
Heated grain mixture*	{ wheat middlings.....25 }	83
	{ yellow corn.....58 }	
Casein†		12
Salt mixture ²		2
Calcium carbonate.....			1
Cod liver oil (medicinal).....			2

*A mixture containing 25 parts of wheat middlings and 58 parts of yellow corn heated to 120°C for one week.

†To 1.2 kg of G.B.I. Vitamin-free Casein or of casein purified by washing with sodium chloride according to the method of either Supplee *et al.*¹¹ or Edgar and collaborators¹², an aqueous solution is added containing 20 mg of thiamin chloride, 20 mg of riboflavin, 20 mg of vitamin B₆ hydrochloride and 1 g of nicotinic acid. The product is thoroughly mixed and then dried at a temperature not exceeding 70°C.

It is similar to the Ansbacher *et al.*⁸ modification of the 240-H ration of Kline *et al.*,⁹ the outstanding difference being the heat treatment of the grain mixture which is prolonged from 36 to about 168 hours.

When baby chicks are fed the heat-treated diet, they grow only very slightly, have an increased blood clotting time and show the typical K-avitaminosis symptoms within from 4 to 8 days, and die within approximately 2 weeks. If it is intended to make vitamin K assays with chicks weighing about 70 g, it is necessary to start baby chicks on a vitamin K-low diet, *e.g.*, Ration K-2,² and to feed Ration K-7 or a similar heat-treated diet after the birds attained the desired weight. Vitamin K-deficiency will occur after the fourth day on the K-7 diet, even when the chicks have access to feces, are housed in dirty cages and receive water contaminated with the diet.

Experiments with chicks have shown that Ration K-7 is not only deficient in vitamin K, but also in pantothenic acid and some other as yet unknown factor or group of factors. Therefore, it may be supplemented with a suitable vitamin B complex concentrate, such as *e.g.* an aqueous extract of rice bran similar to that used by Lepkovsky¹⁰ and known to be rich in pantothenic acid.

Preliminary results brought out the fact that Ration K-7 may be used as the basal diet for rats in studies of the anti-achromotrichia factor. When rats were fed this diet from weaning age on, graying of the hair occurred within 60 to 70 days. The absence of the anti-

⁸ Ansbacher, S., Supplee, G. C., and Bender, R. C., *J. Nutrition*, 1936, **11**, 529.

⁹ Kline, O. L., Keenan, J. A., Elvehjem, C. A., and Hart, E. B., *J. Biol. Chem.*, 1932-33, **99**, 295.

¹⁰ Lepkovsky, S., *Science*, 1938, **87**, 169; *J. Biol. Chem.*, 1938, **124**, 125.

¹¹ Supplee, G. C., Flanigan, G. E., Hanford, Z. M., and Ansbacher, S., *J. Biol. Chem.*, 1936, **113**, 787.

¹² Edgar, C. E., El Sadr, M. M., and Macrae, T. F., *Biochem. J.*, 1938, **32**, 2200.

achromotrichia factor could not be demonstrated, when the same ration without added crystalline vitamin B₆ was used.

It is to be expected that the new vitamin K-deficient diet will be found a useful tool in studies of some of the biological problems involved in vitamin K metabolism, since the diet apparently does not permit a bacterial K-vitamin synthesis.

11419

A Method for Staining of Carious Lesions in Teeth.*

G. GOMORI. (Introduced by R. G. Bloch.)

From the Department of Medicine, University of Chicago.

In the study of experimental rat caries large numbers of animals must be employed to obtain significant statistical results. As a result the methods for examination of the molar teeth become very important. Some investigators have employed the very tedious method of preparing stained thin-sections by the ordinary technics. Others have attempted to reduce the time and expense by resorting to either gross inspection of the carious teeth or to rapid grinding and examination of ground sections. At the suggestion of Dr. B. F. Miller of the University of Chicago the author has developed a rapid, simple and precise method for the staining of carious areas in rat molars (and also in human teeth). Previously,¹ the author had developed a method for the demonstration of insoluble calcium salts in the tissues. It was found that this method cannot be applied to the study of teeth because the silver solution used in the technic will not penetrate the dense dental tissues. Dentin will get a very superficial black coating but enamel is entirely unstained. However, the surprising observation was made that carious areas stained deep black. This can be explained by the greater permeability to the silver solution of the rarefied carious tissue. That the action of acid actually increases the permeability of enamel and dentin was proved by the following experiment: into healthy, extracted human teeth symmetrical holes were drilled, two into each. One of these

* This work has been done under a grant from the Douglas Smith Foundation for Medical Research of the University of Chicago.

¹ Gomori, G., *Am. J. Path.*, 1933, **9**, 253.

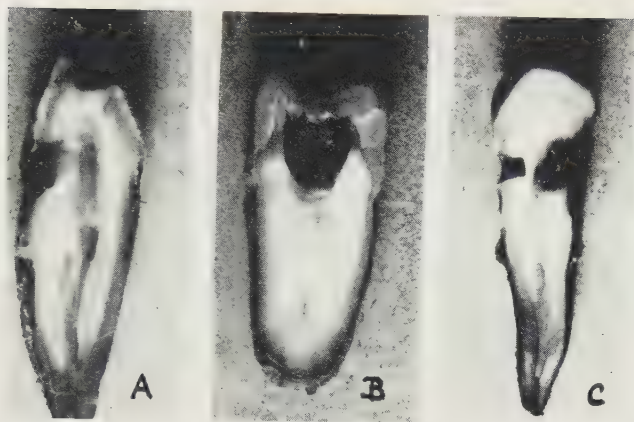


FIG. 1.
Ground Surfaces of Human Teeth.

A. Proximal caries.

B. Secondary caries under a filling.

C. Two holes drilled into a tooth. The left hole, treated with distilled water; the right hole, treated with lactic acid.

holes was packed with cotton soaked in distilled water, the other with cotton soaked in 5 to 10% lactic acid. The holes were sealed with paraffin. The cotton plugs were changed twice daily. After 3 days the plugs were removed, the teeth washed in distilled water and stained according to the technic mentioned. The holes that were treated with distilled water only did not show any staining by silver, whereas the holes packed with lactic acid were surrounded by a black area up to 2 mm in width (Fig. 1).

The silver impregnation method is very suitable for the examination of large numbers of rat jaws for carious lesions. The stained jaws may be directly examined under the surface microscope, or decalcified, embedded in celloidin and made into microscopic sections.

The method is as follows: 1. Fix in neutralized 80 to 95% alcohol or in neutral formalin. 2. Wash tissues in repeated changes of distilled water. 3. Impregnate with a 0.25 to 0.5% solution of silver nitrate for 12 to 24 hours. 4. Wash in many changes of distilled water for at least 24 hours. 5. Reduce in a 5% solution of sodium hypophosphite for 24 hours. 6. Wash under the tap for several hours. 7. Fix in a 2% solution of sodium thiosulfate (hypo) for 12 hours. 8. Wash under the tap for several hours.

The stained jaws may be either dehydrated in alcohol, cleared in cedar oil and examined directly under the dissecting microscope,†

† This variant of the technic has been used by Dr. B. F. Miller (Proc. Soc. Exp. Biol. and Med., 1938, **39**, 389).

or they may be decalcified and embedded in celloidin. Six or more jaws may be included in a single celloidin block. The sections can be counterstained with any stain desired. Sections of several to many dozens of rat jaws depending on the number of sections required, can be cut, stained and mounted in a few hours.

In the finished sections the carious areas are stained deep black (Figs. 2 and 3). A superficial layer of bone and dentin, up to about 20 micra in thickness, will usually be stained black or dark brown, and, in addition, some superficial osteoblasts with their processes. Calcium-containing debris in the fissures will show up as a black, finely granular mass. Healthy enamel is unstained, the exposed

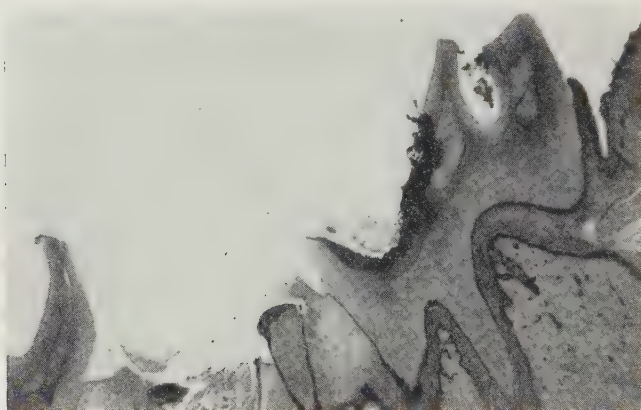


FIG. 2.
Large destructive lesion in a rat molar. $\times 17.5$.

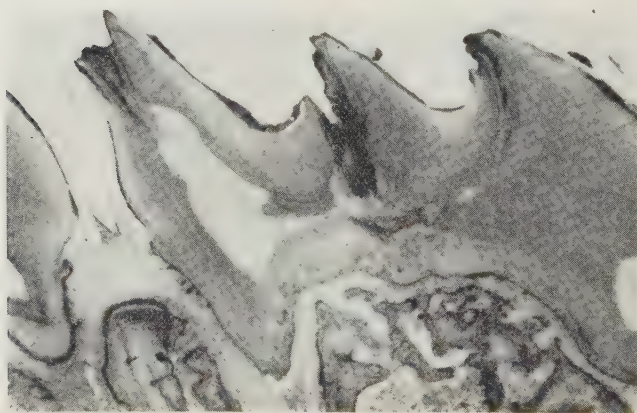


FIG. 3.
Small, deep lesion in a rat molar, starting at the side of a cusp. $\times 35$.

dentin at the tips of the cusps however, often shows a more or less superficial staining. Examination of the sections for lesions is very easy because the carious areas take such an intense, conspicuous black stain.

For decalcification one should employ a 5 to 10% solution of sulfosalicylic acid instead of mineral acids which attack the silver deposits.

The author wishes to express his appreciation to Dr. J. R. Blayney and to Dr. B. F. Miller of the Walter G. Zoller Memorial Dental Clinic for their valuable suggestions.

11420 P

Susceptibility of Field Mice and Meadow Mice to St. Louis Encephalitis.

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In the vicinity of St. Louis the 3 most common species of wild mice are the field mouse, *Reithrodontomys megalotis*, the house mouse *Mus musculus* and the meadow mouse, *Microtus ochrogaster*. As long ago as the epidemic of 1933 efforts were made to trap mice in the homes of encephalitic patients. Several field mice were captured but no representative of either of the other species were obtained in such homes. Beginning in 1934 we tested the susceptibility of field mice to the virus of St. Louis encephalitis and found that they can be infected both by intracerebral and intranasal inoculation. Harford, Sulkin and Bronfenbrenner¹ have reported that the house mouse, *Mus musculus*, is also susceptible to this infection.

More recently we have captured a large number of field mice and also have been able to capture a number of meadow mice. Tests for the susceptibility of these strains of mice to the encephalitic virus have been carried out using simultaneous tests on white Swiss mice for comparison.

¹ Harford, C. G., Sulkin, S. E., and Bronfenbrenner, J., PROC. SOC. EXP. BIOL. AND MED., 1939, **41**, 331.

The effect of the intracerebral injection of 0.030 cc of St. Louis encephalitic virus in dilutions of 10^{-1} , 10^{-2} , 10^{-3} , 10^{-4} , 10^{-5} , 10^{-6} were tried in each species. In the case of the field mice some survivals were noted even in dilutions of 10^{-2} and the majority of the animals survived in dilutions of 10^{-4} , 10^{-5} , and 10^{-6} . In the case of white Swiss mice controls, there were no survivals in less than 10^{-6} . In meadow mice, no survivals were found in dilutions lower than 10^{-4} .

In one experiment using a number of resistant field mice, it was possible to show that animals inoculated intracerebrally with the virus showed no evidence of illness 10 days after the injection, yet were carriers of the virus in their brain tissue since the injection of this brain tissue into Swiss mice regularly resulted in the production of encephalitis. The brain emulsion of the first field mouse was then transferred to a second field mouse. This animal also remained well. Again after a period of 10 days, the brain of the second animal was shown to contain the virus when transferred to white mice. In like manner, the virus was carried through 4 successive transfers in field mice, the brain emulsion producing fatal encephalitis in white mice in each instance.

While we have captured a number of field mice in the homes of encephalitis patients, in no instance have we been able to detect the presence of the virus in the brain, naso-pharynx, spleen or other tissues in any of these animals. We have also examined the tissues of numerous field mice captured at random in various parts of St. Louis and St. Louis County, and in no instance have we been able to show that these animals are carriers of the virus. Field mice and meadow mice while a potential reservoir of encephalitis virus have not been shown to harbor the infection spontaneously.

11421 P

Observations Concerning *Culex pipiens* as a Possible Carrier of St. Louis Encephalitis.

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It has been shown by Casey and Broun that St. Louis encephalitis cases appear to occur with higher incidence in those areas of the city and county which are adjacent to small streams and open ditches.¹ This suggests the possibility of a water breeding insect as a possible transmitting agent. Transmission of the equine types of encephalomyelitis by mosquitoes from animal to animal has been demonstrated by Kelser², Simmons, Reynolds and Cornell,³ and Merrill, Lacaille and Ten Broeck.⁴

Webster, Clow and Bauer⁵ demonstrated the St. Louis encephalitis virus could be taken into the body of the *Anopheles quadrimaculatus* mosquito and retained for the duration of their lives. The virus containing mosquitoes, however, did not infect mice or monkeys by biting. Attempts were made subsequent to the 1933 epidemic to transmit encephalitis from human being to human being by the bite of the various species of mosquito without success.⁶

Since *Culex pipiens* is the most common type of mosquito in the St. Louis area, we have studied the ability of this mosquito to become infected with the virus of St. Louis encephalitis.

Mice infected with St. Louis encephalitis by intraperitoneal injection of heavy doses of virus have been shown by Webster and his co-workers to have a considerable concentration of the virus in the circulating blood for a period of five hours after the injection. In our experiments 1 cc of a 1/10 dilution of virus containing brain was injected intraperitoneally. The mouse was then placed in a specially built biting cage where he was exposed to a number of mosquitoes for a period of 5 hours after dark in a quiet room.

¹ Casey, A. E., and Broun, G. O., *Science*, 1938, **88**, 450.

² Kelser, R. A., Jr., *A. V. M. A.*, 1933, **35**, 767.

³ Simmons, J. S., Reynolds, F. H., and Cornell, V. H., *Am. J. Trop. Med.*, 1936, **16**, 289.

⁴ Merrill, M. H., Lacaille, C. W., and Ten Broeck, Carl, *Science*, 1934, **80**, 251.

⁵ Webster, L. T., Clow, A. D., and Bauer, J. H., *J. Exp. Med.*, 1935, **61**, 479.

⁶ Report on the St. Louis Outbreak of Encephalitis, Public Health Bulletin, No. 214, United States Public Health Service.

At the end of this time the engorged mosquitoes were separated from the unengorged and placed in another cage. Small culture dishes of semi-stagnant water were placed on the floor of the cage to provide a place for the deposition of any eggs that might be laid and as a moisture source for the mosquitoes.

A number of engorged mosquitoes were selected at random and killed in ether fumes. Usually 3 mosquitoes were ground in a mortar and diluted with 10 cc of salt free broth, and then passed through a Berkefeld filter. Serial dilutions of 1:5, 1:10, 1:100 were made of the filtrates and each injected intracerebrally into 3 Swiss mice in 0.03 cc amounts. The injected animals were observed for 15 days after injection.

In 29 series of experiments in which the mosquitoes which previously had engorged on infected mice were macerated and injected into normal mice, some of the injected mice died in 10 instances. In only 3 cases, however, were we able to carry the virus in serial transfers and to prove conclusively by neutralization tests that the virus of St. Louis encephalitis had been the infecting agent.

In the successful experiments we have so far shown survival of the virus in the body of the mosquito no longer than 10 days. The concentration of the virus within the body of the mosquito has not been shown to be greater than 100 intracerebral lethal mouse doses. This is a very much smaller concentration than was shown by Webster to be present in the body of the *Anopheles* mosquito.⁵

Two series of experiments were conducted in which larvæ of about the first or second instar were placed in water containing a macerated infected brain suspension. The dilution of the brain suspension in water was 1:10. When these larvæ transformed into the adult form, the mosquitoes were macerated in broth, filtered and injected into normal mice. None of the mice showed any evidence of infection after such injections.

Nine series of experiments were conducted in which eggs laid by mosquitoes which had previously been allowed to feed on infected mice were macerated in broth, filtered and injected into normal mice.⁴ In all cases the mice remained normal and healthy.

In 15 experiments normal mice were exposed in the biting cages to 50 to 100 mosquitoes which had previously been allowed to feed on an infected mouse and then allowed to stand until they became empty. They were then observed until they had been allowed to feed on the normal mice. The mice were allowed to remain in the cage for 12 hours and then removed. In all cases the mice were healthy and normal after 15 days.

Validity of Chemical Balance Studies in Eviscerated Animals, as Index of Carbohydrate Utilization.

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We have recently employed the chemical balance method in the abdominally eviscerated animal, to study the utilization of carbohydrate by the peripheral tissues of dogs under the following conditions (a) normal and depancreatized;¹ (b) normals treated with insulin;^{2,3} (c) normals treated with an anterior pituitary extract;⁴ (d) hypophysectomized;^{4,5} (e) phlorhidzinized.⁶ The chemical balance was struck from the blood sugar, blood lactic acid and the muscle glycogen values at the beginning and end of the experiments; and from the amount of sugar administered during the experiment. The calculations from these data were based upon distribution ratios reported by others, namely, 1/6 of the body weight for sugar and lactic acid^{7,8} and 1/2 of the body weight for muscle glycogen.^{9,10}

There are 2 possible objections to the manner in which we calculated the utilization of carbohydrate by the muscles: (1) The possible presence of significant amounts of free sugar or of higher carbohydrate intermediates in the muscles, which would not be taken into account by glycogen estimations alone. (2) The possible presence of amounts of lactic acid in the muscles, which are not in equilibrium with the blood values. We have therefore repeated utilization experiments on eviscerated normal and depancreatized

* Aided by the Max Pam Fund for Metabolic Research.

¹ Soskin, S., and Levine, R., *Am. J. Physiol.*, 1937, **120**, 761.

² Soskin, S., and Levine, R., *Am. J. Physiol.*, 1938, **123**, 192.

³ Soskin, S., and Levine, R., *Am. J. Physiol.*, 1940, in press.

⁴ Soskin, S., Levine, R., and Lehmann, W., *Am. J. Physiol.*, 1939, **127**, 463.

⁵ Soskin, S., Levine, R., and Heller, R. E., *PROC. SOC. EXP. BIOL. AND MED.*, 1938, **38**, 6.

⁶ Soskin, S., Levine, R., and Lehmann, W., *PROC. SOC. EXP. BIOL. AND MED.*, 1938, **39**, 442.

⁷ Burn, J. H., and Dale, H. H., *J. Physiol.*, 1924, **59**, 164.

⁸ Lavietes, P. H., Bourdillon, J., and Klinghoffer, K., *J. Clin. Invest.*, 1936, **15**, 261.

⁹ Best, C. H., Dale, H. H., Hoet, J. P., and Marks, H. P., *Proc. Royal Soc. London B*, 1926, **100**, 55.

¹⁰ Dye, J. A., and Chidsey, J. L., *Am. J. Physiol.*, 1939, **126**, P482.

NORMAL DOG, EXP. 1.

Wt 11.4 kg. Exp. period 4 hr. Avg blood sugar throughout exp. was 92 mg%. Muscle mass calculated as 50% of body wt equals 5700 g. In calculation (A) the blood and extracellular fluid is taken as $\frac{1}{4}$ of the body wt, 7.8 equal to 1900 cc. In calculation (B), the blood and extracellular fluid is considered equal to 30% of body wt, 13.14 Blood volume equals 7%. Extracellular fluid of muscle (30 — 7)/2 equals 11.5%. Blood plus non-muscle extracellular fluid equals 18.5%, or 2109 cc.

Calculation (A)		Calculation (B)	
159×5700	= 9063 mg	159×5700	= 9063 mg
100		100	
Total CHO decrease		Total CHO decrease	
Initial blood sugar	84.0 "	Initial blood sugar	84.0 "
Final	122.0 "	Final	122.0 "
	Diff. 38.0 "		Diff. 38.0 "
Increase in extracellular sugar		Increase in extracellular sugar	
Initial blood lactic acid	32.4 "	Initial blood lactic acid	32.4 "
Final	129.0 "	Final	129.0 "
	Diff. 96.6 "		Diff. 96.6 "
Increase in extracellular lactic acid		Increase in extracellular lactic acid	
Initial muscle lactic acid	37.4 "	Initial muscle lactic acid	37.4 "
Final	72.6 "	Final	72.6 "
	Diff. 35.2 "		Diff. 35.2 "
Increase in muscle lactic acid		Increase in muscle lactic acid	
Dextrose injected during experiment		Dextrose injected during experiment	
Total sugar disposed of	5455 mg	Total sugar disposed of	5455 "
Total retained sugar	(9063 + 5455) = 14518 "	Total retained sugar	(9063 + 5455) = 14518 "
Sugar utilized	(722 + 1835) = 2557 "	Sugar utilized	(802 + 2038 + 2006) = 4846 "
	(14518 — 2557) = 11961 "		(14518 — 4846) = 9672 "
11961		9672	
11961	= 262 mg	9672	= 212 "
11.4×4		11.4×4	

TABLE I.
Summary of Data for Alternative Calculations of Carbohydrate Utilization by Exsuscated Normal and Deapaneritized Dogs Respectively.

Dog	No.	Wt, kg	Duration of exp.		Total sugar injected, g	Blood sugar		Blood lactic acid		Total CHO in muscle		Lactic acid in muscle		
			Hr	Min		Initial, Final, mg %	Avg, mg %	Initial, Final, mg %	mg %	Initial, Final, mg %	mg %	Initial, Final, mg %	mg %	
Norm.	1	11.4	4	0	5.46	84	122	92	32.4	129.0	449.9	290.3	37.4	72.6
"	2	9.7	4	0	6.97	236	277	262	47.2	125.0	461.7	264.0	12.9	56.3
"	3	9.4	3	0	9.50	474	675	574	32.4	61.0	581.5	399.5	25.0	34.5
Dep.	4	8.4	3	0	1.60	205	175	193	51.2	111.6	470.0	392.0	97.0	98.0
"	5	12.5	3	0	14.90	337	646	506	132.8	160.0	431.6	387.3	154.8	102.2
"	6	7.4	2	40	1.59	390	392	403	77.0	204.0	443.0	207.0	54.2	148.6

dogs respectively, in which we have determined the total carbohydrate content of the muscle instead of muscle glycogen, and the lactic acid content of the muscle as well as blood lactic acid. Our results by these methods are presented, and are compared with our previous results.

Methods and Results. The details regarding the technic of these experiments and the chemical methods employed were described in a previous publication.¹ The total carbohydrate estimations in the present work were made by the method of Tsai, as modified by Benoy and Elliott.¹¹ The blood and muscle lactic acids were determined by a slight modification of the method of Miller and Muntz.¹²

The data are summarized in Table I. Calculations of utilization from these data were made in 2 ways: (A) depending on the distribution ratios used in our previous work, (B) treating blood and muscle separately. These 2 types of calculation are exemplified in parallel, for animal No. 1.

It may be seen that the results of the above calculations, although they show some difference, are of the same order of magnitude. Fig. 1 graphically represents the results of both calculations for all our present animals, and offers a comparison with our previously published results and calculations. The present methods of calculating introduce only a slight and consistent difference in the data as a whole. It is understood, of course, that none of the above methods can be held to yield absolute utilization values. But it is equally evident that any of them give good comparative results.

Discussion and Summary. It is clear that neither of the possible objections to our previous method of calculating carbohydrate utilization is sufficiently valid to materially affect the end results. That is, the amounts of free sugar, higher carbohydrate intermediates, or lactic acid present in the muscles are not such as to invalidate calculations based on blood sugar and lactic acid values, and distribution ratios. This agrees with the earlier, basic work of Best, Dale, Hoet and Marks,⁹ who demonstrated that the sugar which disappeared from the blood of eviscerated spinal cats was equal to the sum of the glycogen deposited in the muscles and the glucose equivalent of the oxygen consumed.

The close correspondence of utilization rates, as determined by

¹¹ Benoy, M. P., and Elliott, K. A. C., *Biochem. J.*, 1937, **31**, 1268.

¹² Miller, B. F., and Muntz, J. A., *J. Biol. Chem.*, 1938, **126**, 413.

¹³ Chidsey, J. L., and Dye, J. A., *Am. J. Physiol.*, 1939, **126**, P461.

¹⁴ Peters, J. P., *Body Water*, Springfield, C. C. Thomas, 1935, p. 138.

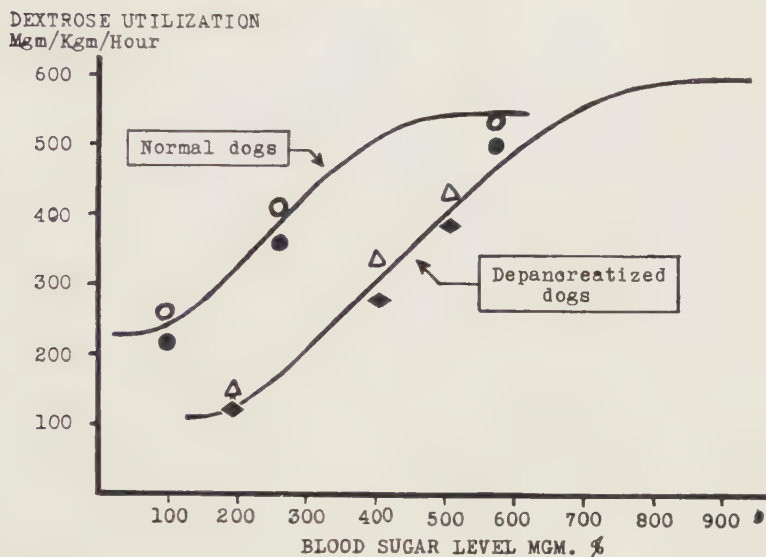


FIG. 1.

Comparison of Carbohydrate Utilization by Normal and Depancreatized Dogs by Different Calculations.

The plotted points represent the results of the present work and calculations, and are *not* the points through which the smooth curves are drawn. The curves are included for comparison, and are derived from previous work¹ on the rates of sugar utilization by the extra-hepatic tissues of normal and depancreatized dogs, respectively.

- Normal dogs, calculation (A).
- Normal dogs, calculation (B).
- △ Depancreatized dogs, calculation (A).
- ◆ Depancreatized dogs, calculation (B).

different methods and by different calculations, strongly supports the validity of the chemical balance method as a means of determining carbohydrate utilization in liverless animals.

Vitamin C Requirement of the Guinea Pig.*

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The assumed daily requirement of ascorbic acid for the guinea pig has gradually been increased from 0.5 mg to 1.5 mg (Szent-Györgyi.¹) In fact, Zilva² showed that even on a dosage of 1.5 mg per day saturation of the tissues did not occur, and that about 20 times this amount was necessary to maintain saturation of the tissues. However, since Zilva's animals apparently thrived on 1.5 mg per day, this author assumed that this amount sufficed to assure undisturbed growth and health.

Szent-Györgyi,¹ however, believes that the true physiologic daily dose of the vitamin is that amount which the animal consumes in its native environment. He calculated that in his natural habitat the guinea pig ingests about 30 mg of ascorbic acid daily, and that this amount compares favorably with the amount needed to maintain a maximal saturation of its tissues. During the course of the experiments on the relationship of vitamin C to complementary activity,³⁻⁵ 870 blood-serum ascorbic-acid determinations were made. The guinea pigs were given varying amounts of ascorbic acid.

This report deals with the relationship of the blood-serum ascorbic-acid level to the daily intake of varying amounts of the vitamin.

Methods. All the animals were maintained on a stock diet of Purina mixed rabbit chow. This mixture is supposed to contain all the essential elements needed for full growth of the animals with the exception of vitamin C. In fact, guinea pigs develop scurvy when maintained on this diet for periods longer than 21 days.

Crystalline ascorbic acid was administered either as a commercial sodium salt known as "Cevalin" or as ascorbic acid neutralized

* Aided by a grant from the Commonwealth Fund.

¹ Szent-Györgyi, A., *Deutsch. med. Wchnschr.*, 1937, **63**, 1789.

² Zilva, S. S., *Biochem. J.*, 1936, **30**, 1419.

³ Ecker, E. E., Pillemer, L., Wertheimer, D., and Gradis, H., *J. Immunol.*, 1938, **34**, 19.

⁴ Pillemer, L., Seifter, J., Kuehn, A. O., and Ecker, E. E., to be published.

⁵ MacDonald, F., and Johnson, H., to be published.

before injection with 0.1 N NaOH. All injections were given intramuscularly.

A modified Tillman's procedure was used for the estimation of the ascorbic-acid content of the serum. The method follows: 4 to 5 cc of blood were drawn by cardiac puncture and allowed to stand for 15 minutes and centrifugalized. Two cc of the serum was then added to 4 cc of freshly prepared 20% trichloroacetic acid. The mixture was allowed to stand for 5 minutes and again centrifugalized. Three cc of the clear supernatant liquid was then titrated against 2,6-dichloro-phenolindophenol (Hoffbann-LaRoches tablets). Each cc of the dye solution is equivalent to 0.02 mg of ascorbic acid. All titrations were done in duplicate and triple distilled water was always employed.

TABLE I.
Daily Dose of Ascorbic Acid and Blood-serum Ascorbic Acid in the Guinea Pig.

No. of determinations	Daily dose, mg	Blood-serum ascorbic acid in mg%		
		min.	max.	med.
40	0	0	.05	.02
220	0.5	0	.21	.09
218	1.0	.05	.36	.13
47	2.0	.12	.52	.33
117	5.0	.39	.74	.57
143	10.0	.83	1.44	1.03
85	20.0	.87	1.73	1.08

Results. The table gives a composite protocol of the results of 870 blood-serum ascorbic-acid values.

It is seen that the maximal concentration of vitamin C in the serum of the guinea pig only occurs when a daily dose of from 10 to 20 mg is given. Since it has been shown that serum-complement reaches its maximal activity at a level of about 1 mg of ascorbic acid per 100 cc, it is suggested that an amount of 10 mg per day be given to maintain the normal activity of the blood-serum proteins since a full complementary activity is an expression of the normal behavior of these proteins. Cohen⁶ found that 5 mg of ascorbic acid per day was needed in a tooth-protective ration.

Summary. Analysis of a series of 870 blood ascorbic-acid values reveals that a daily intake of from 10-20 mg of ascorbic acid is needed to secure a 1 mg per 100 cc concentration in the blood serum of the guinea pig. Under these conditions a normal complement-value is obtained.

⁶ Cohen, M. B., *J. Allergy*, 1938, **10**, 15.

Dialysis of Complement Against 1.0 Normal Sodium Chloride.*

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Chow and Zia¹ reported that when guinea-pig serum is dialyzed against normal NaCl solution through a cellophane membrane at about 5°C its complementary activity rapidly decreases. They also stated that they were able to restore practically all of the original activity of the serum by combining the dialysate with the non-dialyzable fraction, and concluded that the loss of activity was due to the removal of a dialyzable component of the complement. Since these authors were unable to reactivate dialyzed serum by the addition of yeast-inactivated complement, or ammonia-inactivated complement, or by complement inactivated by oxidation with iodine, but were able to reactivate with dialysates of serums which had previously been inactivated by any of the above methods, they state that "these results, therefore, indicate that the dialyzable component of complement is different from the third or fourth or the oxidizable components of complement."

Because of the introduction of a new factor or factors in the constitution of complement it was thought of interest to study the reactivation of complement which had been inactivated by dialysis. However, upon repetition of the experiments of Chow and Zia, it was not possible to confirm their results in respect to the inactivation of complement by dialysis under the conditions reported by them.

It was observed that serum dialyzed in the cold (4.5°C) against 1.0 N NaCl, even over long periods of time, loses its hemolytic activity no more rapidly than serum which is stored without dialysis under the same conditions.

Experimental. Five cc of fresh guinea pig serum were measured into a 5/8-inch cellophane tube (No. 341), the bottom of which had been folded and tied off with silk thread in such a way as to prevent leaking. The top of the tube was sealed with a rubber stopper and tightened with a rubber band. The cellophane sac containing the

* Aided by a grant from the Commonwealth Fund.

¹ Chow, B. F., and Zia, S. H., *Proc. Soc. Exp. Biol. and Med.*, 1938, **38**, 695.

serum was then suspended in a large stoppered test tube containing 50 cc of cold 1.0 N NaCl solution. The sac was suspended in the saline at such a height that the level of the saline was about a centimeter above the level of the serum within the sac. A control sample of the same guinea-pig serum was measured into a similar cellophane sac and sealed in the same manner as above. This was suspended in an empty, large, stoppered test tube.

The large test tubes containing the sacs were placed in a cold room at 4.5°C. At the end of 24 hours, the sacs were opened and 0.05 cc of serum was removed from each sac. These samples were diluted 1:30 with 0.9% NaCl solution and the complement-activity of each sample was determined by the method of initial hemolysis of Ecker *et al.*² After removal of the samples for titration, the cellophane sacs were again sealed. Each serum undergoing dialysis was suspended in another 50 cc portion of cold 1.0 N NaCl solution. Dialysis was allowed to proceed at 4.5°C for another 24-hour period at the end of which time the above procedure was repeated. This was continued throughout the course of the experiment. The volume of each dialyzed serum and of the control serum was measured at intervals during the experiment. It was found that in no case did the volume increase by as much as 10% during the entire experiment. There was no change in the volume of the control.

The table shows the complement values obtained for three dialyses of 2 different serums, and also the titration values for the controls.

Conclusions. It is seen from the above table that guinea-pig serum slowly loses some of its complementary activity during dialysis against 1.0 N NaCl solution at a low temperature over a period of 2 weeks. This loss in activity is in no way comparable, however, with that reported by Chow and Zia who stated that in a

TABLE I.
Complement-activity of Serum During Dialysis in the Cold Against 1.0 N Sodium-chloride Solution.

Period of dialysis in hr	0	24	48	72	96	144	168	192	216	240	264
Dialyzed serum A	.03	.04	.03—	.04—	.04+	.04		.08		.08	
Control A	.03	.03	.03+	.04—	.04+	.04		.07+		.08	
Dialyzed serum B ₁	.02	.03	.03	.04—	.04		.03		.04		.03
Dialyzed serum B ₂	.02	.03	.03	.03	.04		.03		.04		.03
Control B	.02	.03	.03	.03	.04		.03		.04		.03

² Ecker, E. E., Pillemer, L., Wertheimer, D., and Gradis, H., *J. Immunol.*, 1938, **34**, 19.

typical experiment a sample of serum lost nearly 90% of its original activity upon dialysis for 4 days under the same experimental conditions, while a control sample lost only 25% of its activity during storage for the same period. It will be noted from the results of the present experiments that both the total loss and the rate of loss of complement activity in the dialyzed serum is in each case substantially identical with that of the undialyzed control serum. It is upon this evidence that the conclusion is drawn that none of the components of complement are removed from the serum by dialysis against 1.0 N NaCl. The loss of complementary activity obtained by allowing serum to remain in the cold for long periods of time may be explained upon the basis of oxidation alone, or other factors, since neither complement nor any of its parts is dialyzable through a cellophane membrane against 1.0 N NaCl solution in the cold. In additional experiments, little loss of activity was obtained in dialyzing against cold 1.0 N NaCl solution complement which had been purified by a fractional precipitation method which will be reported in a subsequent paper. Dialysis in this case has been allowed to proceed for periods as long as 10 days.

Summary. Dialysis against 1.0 N NaCl at 4.5°C does not remove a dialyzable component from the guinea-pig complement. The gradual loss of activity noted may be accounted for on the basis of changes within the protein-molecule.

11425

Butter Fat in Dermatitis-Producing Diets.

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Butter fat has been revealed both as a curative agent¹ for rat dermatitis and also as a component (9%) of a dermatitis-producing diet.² The curative property was demonstrated by a daily supplement of 500 mg of fresh butter fat, a quantity approximately equal

* Present address: Hospital of the Rockefeller Institute for Medical Research, New York.

¹ Schneider, H. A., Ascham, J. K., Platz, B. R. and Steenbock, H., *J. Nutr.*, 1939, **18**, 99.

² György, P., *Biochem. J.*, 1935, **29**, 741.

to the amount consumed by the rat when the butter fat is incorporated in the diet at a level of 9%. Butter fat is thus placed in the peculiar position of being an anti-dermatitis agent when fed as a supplement, and of failing to exhibit this property when mixed with the diet. This anomalous rôle of butter fat was clarified by the following experiments. These indicate that the mixing of butter fat in the diet may result in a destruction of the anti-dermatitis potency of the former when the mixed diet is allowed to stand in contact with the air for periods of time usually met with in the laboratory production of rat dermatitis.

Experimental. The diet used had the following composition. Glucose 68, casein (alcohol extracted) 18, salts 4, cod liver oil 1, butter fat 9. In addition each rat received the following daily supplements: 5 μ g calciferol and 10 μ g beta carotene in 1 drop of the liquid fraction of hydrogenated coconut oil, plus 20 μ g riboflavin and 10 μ g thiamin chloride hydrochloride in 1 drop of N/50 acetic acid. The diet, apart from vitamin supplements, was fed in two forms, "fresh" and "rancid." The "fresh" diet was made up weekly, previously prepared but unused quantities being discarded, and was stored in a refrigerator between feedings. The "rancid" diet was made up one month before use and was stored for that period, open to the air, in a warm room. When used the "rancid" diet had the characteristic odor of rancidity (Peroxide No. of ether extracted fat was 131). Diets were fed daily and uneaten portions discarded.

The rats used were 35-40 g weanlings specially prepared³ on a diet low in anti-dermatitis factors. When 3 rats were fed the "rancid" diet they developed a florid dermatitis in 6 weeks. Three rats fed the "fresh" diet failed to develop any symptoms of dermatitis even after 15 weeks. When the rats which had developed dermatitis on the "rancid" diet were changed to the "fresh" diet, the dermatitis was cured in three weeks. When the experiment was repeated with the same number of animals using the paired feeding method the results were the same. The differences thus observed could not be attributed to differences in consumption of the diets.

Summary. The anti-dermatitis action of fresh butter fat has been confirmed.

Destruction of the anti-dermatitis potency of butter fat has been demonstrated in a diet in which the butter fat was allowed to become rancid.

³ Quackenbush, F. W., Platz, B. R., and Steenbock, H., *J. Nutr.* 1939, **17**, 115.

Bacteriostatic Effect of Sulfathiazol and Sulfamethylthiazol for Beta Hemolytic Streptococci in Tissue Culture Clots.*

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The bacteriostatic effect of 2(para-amino-benzene-sulfonamido)thiazol (sulfathiazol) and 2(para-amino-benzene-sulfonamido)4-methylthiazol (sulfamethylthiazol) has been compared with that of sulfanilamide. Two strains of beta streptococci have been used, C 203 and No. 40. Strain C 203 has been used in many studies reported in the literature. Strain No. 40 is a Lancefield group C of human origin which has been studied extensively in this laboratory.

The Maximow culture technic was used. Details have been described previously.¹⁻⁴ A culture consists of one drop of heparinized rabbit plasma and 3 drops of rabbit serum extract of 7-day chick embryos.

The extract was inoculated with a culture of streptococci grown in the serum extract described above to which 5% of rabbit erythrocytes were added. Cultures are grown only until hemolysis occurs. For strain No. 40 this requires 3-5 hours and somewhat longer for C 203. Dilution of the bacterial culture was made rapidly through Tyrode into the extract to make a final dilution of 10^{-6} . The inoculated extract was then divided into 4 equal portions and equal volumes of the designated drug added to each of the experimental tubes and the same volume of Tyrode to the control.

A 400 mg % stock solution of each drug was sterilized by Berkefeld filtration through a filter used only for that drug. Solutions were kept in the ice box.

Due to the limited solubility of the thiazol derivatives these were used as the sodium salts.

* Aided by grants from the Medical Research Fund, Graduate School, the University of Minnesota, and the Department of Medical Research, the Winthrop Chemical Co., Inc. All drugs supplied by the Winthrop Chemical Co., Inc. Assistance in the preparation of these materials was furnished by the personnel of the Work Projects Administration, official project No. 65-1-71-140, sub-project No. 237, and by the National Youth Administration.

¹ King, J. T., *Arch. f. Exp. Zellforsch.*, 1930, **9**, 341.

² King, J. T., *Arch. f. Exp. Zellforsch.*, 1931, **10**, 467.

³ King, J. T., *Arch. f. Exp. Zellforsch.*, 1937, **20**, 208.

⁴ King, J. T., Henschel, A. F., and Green, B. S., *J. Am. Med. Assn.*, 1939, **113**, 1704.

pH determinations done with the glass electrode show that, in the amounts used, the change in pH caused by addition of the sodium salts is small.

Cultures were incubated at 37.5°C.

Readings were made at magnification of 60X with a standardized ocular micrometer using a mechanical stage (114 units = 1 mm). The average colony diameter was used as the index of bacteriostasis. Colony counts per culture are also given in the table.

The effect of 3 concentrations of each drug was studied for each strain. With one exception readings were made at or near the 24-hour period. The results are given in Table I.

It will be noted that even on the weight basis sulfathiazol compares favorably with sulfanilamide in inhibiting the growth of both strains studied. Sulfamethylthiazol is somewhat less effective.

When used in sufficient concentration to cause marked bacteriostasis, both drugs inhibit the development of the normal diffuse periphery usually seen around colonies growing in this medium. The periphery may be completely inhibited or it may be composed of a loose net of long, heavy chains of streptococci which are never seen in the controls. This influence on the periphery has been described previously for sulfanilamide in this medium by King, Henschel and Green.⁴ Others have noted the formation of such chains in fluid media.

Sulfamethylthiazol is less effective than sulfathiazol in inhibiting the development of the normal diffuse periphery.

Strains which we have studied previously have usually not shown a significant decrease in the number of colonies when growing in tissue culture clots containing sulfanilamide. In the highest con-

TABLE I.
Action of Sulfanilamide (S), Sulfathiazol (ST), and Sulfamethylthiazol (SMT)
on Two Strains of Beta Streptococci; 22-24 Hours Except as Noted.

Drug conc. mg%	50		10		1	
	No. Cols.	Diam.	No. Cols.	Diam.	No. Cols.	Diam.
Strain C203.						
Control	44.2	60.8	40.7	38.7	23.1	45.1
S	5.2	5.6	20.2	20.9	18.6	42.3
ST	2.5	5.6	22.2	15.6	23.5	49.2
SMT	16.2	11.6	31.0	24.8	20.2	49.0
Strain No. 40.						
Control	24.7	77.7	84.2	73.4*	53.6	85.4
S	14.5	9.4	85.2	22.8	52.3	57.2
ST	6.2	5.8	89.3	19.7	53.6	72.0
SMT	8.0	8.4	92.3	26.6	50.6	83.4

* 10 mg% conc. read at 48 hours.

centration of the drugs used in this study, strain No. 40 did show a decrease in colony count at 24 hours. By 48 hours, however, the counts had increased to 22.5 in cultures containing sulfanilamide, 20.5 in sulfathiazol and 21.7 in sulfamethylthiazol (controls 24.7).

Strain C 203, which we are using for the first time, shows a decrease in colony count at 24 hours not only in the 50 mg% concentration but also in 10 mg%. Furthermore, the count in the 10 mg% concentration had increased to only 32.7 in sulfanilamide, 31.0 in sulfathiazol and 38.7 in sulfamethylthiazol at 72 hours (control 40.7). In the 50 mg concentration there was no significant increase in average number of colonies per culture at 5 days. Some of the few colonies which were present continued to grow very slowly, however. This strain is definitely more susceptible in this respect than strains which we have studied previously.

Due to differences in method of assay and drug-organism combinations, it is difficult at this time to compare the results of *in vitro* tests. It is now generally realized that the medium used and the size of the inoculum markedly influence the results obtained.

Lawrence⁵ found sulfathiazol and sulfamethylthiazol superior to sulfanilamide in their bacteriostatic effect against group A hemolytic streptococci. Long and Bliss⁶ found sulfathiazol to be as effective as sulfanilamide against a number of organisms including group A streptococci.

Summary. Under the experimental conditions employed sulfathiazol is as effective a bacteriostatic agent against the strains of beta hemolytic streptococci tested as is sulfanilamide. Sulfamethylthiazol is somewhat less effective.

⁵ Lawrence, C. A., PROC. SOC. EXP. BIOL. AND MED., 1940, **43**, 92.

⁶ Long, P. H., and Bliss, E. A., PROC. SOC. EXP. BIOL. AND MED., 1940, **43**, 324.

11427

Growth of Bacteria in Media Containing Colchicine.

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Colchicine is toxic for plant and animal cells, and seems to be specifically a mitotic poison, preventing normal division of the chromosomes and thus causing mutation.¹ True bacterial cells differ from the cells of the higher plants and animals, in that so far as is known they do not contain chromosomes or well defined nuclei.

If the above statements are true then we might expect that colchicine would have either no effect or a different effect on bacterial cells than on the cells of the higher plants.

This work was designed to study the effect of colchicine on several different types and species of bacteria with respect to the character of growth, colony characteristics, cell morphology, biochemical reactions, and acceleration or inhibition of multiplication of the organisms.

Methods. A 4% solution of colchicine in plain meat infusion broth was sterilized by filtration through a Berkefeld N filter. This solution of colchicine was then diluted with broth to the desired concentrations. All media used were adjusted to pH 7.2.

Serial Transfers in Plain Broth and Colchicine Broth. One standard 4-mm loopful of a 24-hour broth culture of *Staphylococcus H* was inoculated into 5 cc of a 2% colchicine broth and into the same amount of plain broth. A culture of *B. typhosus* 109 was inoculated in the same manner. All cultures were incubated at 37°C. Serial transfers were made daily into fresh media of the same kind (colchicine into colchicine, and plain broth into plain broth) for 12 days and every 48 hours thereafter until a total period of 22 days had elapsed.

At the time of each transfer subcultures were made on plain agar plates in order to observe colony morphology and also smears made and stained by Gram's method. On the 8th and 16th transfer all the cultures were inoculated into various carbohydrate media, milk and gelatin for observations of their biochemical reactions.

Old Cultures. Cultures of *Staphylococcus aureus* H and *B.*

¹ Nebel, B. R., and Ruttle, M. L., *J. Heredity*, 1938, **29**, 2.

typhosus 109 were made in 2% colchicine and plain broth as before but instead of serial transfers the original cultures were incubated at 37°C for 22 days during which time subcultures were made every 4 days and treated in the same manner as described above.

Serial Transfers on Plain and Colchicine Agar. Plain agar and 2% colchicine agar plates were inoculated with a 24-hour broth culture of *Staphylococcus aureus* H and *B. typhosus* 109 in such a manner as to obtain isolated colonies. After 48 hours' incubation single colonies were picked and re-streaked on fresh plates. The colonies from the colchicine agar plates were streaked on colchicine agar and also on plain agar plates, and the colonies from the plain agar plates were streaked on plain agar and also on colchicine agar plates. These transfers were made every 48 hours over a total period of 22 days. Smears were made of the colonies and stained by Gram's method.

Other Organisms. A second series of colchicine broth tubes was inoculated using in this series varying concentrations of colchicine (2%, 1%, 0.50% and 0.25%). The organisms used were *Staphylococcus aureus* 1038, *Staphylococcus aureus* L, *Streptococcus hemolyticus*, *M. catarrhalis* and *B. megatherium*. Control tubes of plain broth were inoculated at the same time. Incubation was at 37°C for 22 days with the following observation at 48-hour intervals for the first 6 days and at 4-day intervals thereafter. Subcultures were made on plain agar plates for observations of colonies, smears made and stained by Gram's for morphology, and inoculation into various carbohydrate media, gelatin and milk to note changes, if any, in cultural reactions. Comparison was made of the amount of growth in the colchicine broth with that in plain broth controls as an indication of any inhibiting or accelerating action of the colchicine on the growth of the organism.

Results. The type of growth of *Staphylococcus aureus* H in 2% colchicine broth was markedly different, being a coarse granular growth which settled out rapidly, in contrast to the diffuse turbid growth of the plain broth controls. On 2% colchicine agar the colonies were wrinkled, waxy, and of a peculiar greenish-yellow color. The cell morphology in the colchicine broth and agar differed from the controls in that the organisms were larger and arranged in tetrads and had the appearance of a stained preparation of *sarcinae*. These changes in growth, colony and cell morphology occurred on the first transfer to media containing colchicine and could be obtained only when grown in the presence of colchicine. On transfer to plain media the organisms immediately reverted to normal.

There was no change in the biochemical reactions. All the other organisms used, including 2 other strains of *Staphylococcus*, showed no variation from normal when grown in the presence of colchicine. The growth of *Streptococcus hemolyticus* was inhibited by 1% of colchicine; 0.50% and 0.25% had no apparent effect. There was marked but not complete inhibition of growth of *M. catarrhalis* in 2% colchicine, some inhibition in 1% but none in 0.50% and 0.25%. The staphylococci and *B. megatherium* grew equally well in all concentrations of colchicine used. There was no evidence of any stimulating effect on the growth of any of the organisms.

Blakeslee² says that colchicine affects only the cell division of the chlorophyl-bearing plants and that the fungi including the bacteria are not affected. Jennison³ could detect no change in the rate of reproduction or colony morphology of bacteria in the presence of colchicine. With the exception of one strain of *Staphylococcus* which showed a temporary variation in cell and colony morphology when grown in the presence of colchicine, our results are in accord with these observations but further indicate that colchicine does not affect the cell metabolism of bacteria.

11428

The Liver and Endogenous Androgens.*

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It is well known that most androgens and estrogens are either inactive when administered orally, or are less potent than when administered subcutaneously. In the case of the estrogens, experimental evidence has been offered to explain this phenomenon. All workers agree that the lack of effectiveness with oral administration is due to inactivation of estrogens in the liver. When natural estrogens are incubated *in vitro* with liver,^{1,2} their potency is lost.

² Blakeslee, A. F., *Science*, 1939, **89**, 10.

³ Jennison, M. W., *J. Bact.*, 1940, **39**, 20.

* Supported in part by a grant from the Josiah Macy, Jr., Foundation.

¹ Zondek, B., *Skand. Arch. f. Phys.*, 1934, **70**, 133.

² Heller, C. G., *Endocrin.*, 1940, **26**, 619.

When ovaries are implanted intra-mesenterically so that the venous drainage is through the liver, the animal manifests changes characteristic of castration but, when the same ovary is later regrafted into the axillary region, the castration effects disappear.³ Talbot⁴ showed that impairment of liver function by CCl_4 apparently increased the concentration of endogenous estrogen in the blood stream as evidenced by increase in uterine weight.

Concerning the possible inactivation of androgens by the liver, only two reports have been offered. Biskind and Mark⁵ showed that pellets of testosterone propionate are ineffectual when implanted into the intact spleen, but not when implanted into the spleen after the original blood supply of this organ has been altered. This indicates that inactivation of the testosterone propionate occurs only when it passes from the site of absorption directly through the portal system. Biskind,⁶ by the same type of experiment, showed that methyl testosterone is also inactivated when it passes first through the portal system. In view of the known oral effectiveness of methyl testosterone, in contrast to all other androgens, it seems probable that this substance is removed from the gastro-intestinal tract by some route other than the portal system.

The present report is concerned with the possible rôle of the liver in the inactivation of testicular androgens. The transplantation method devised by Golden and Sevringhaus was used.

Twenty-five males, 20 to 23 days old, were castrated and, in each case, one-half of a testis was sutured into the gastro-splenic mesentery. At the same time 21 males of the same age were castrated and one-half a testis was implanted subcutaneously into the groin of each.

One or two months after implantation the surviving animals were killed. In each case the size and appearance of the penis was noted, the general character and vascular relationships of the implant were established, and the ventral prostates and seminal vesicles were removed and weighed. In some of the animals the thymus was also weighed. It was thought that the thymus might present an additional check for the presence or absence of androgens, inasmuch as thymic hypertrophy after castration has been mentioned in the literature.

³ Golden, June B., and Sevringhaus, E. L., *Proc. Soc. Exp. Biol. and Med.*, 1938, **39**, 361.

⁴ Talbot, N. B., *Endocrin.*, 1939, **25**, 601.

⁵ Biskind, G. R., and Mark, Jerome, *Bull. Johns Hopkins Hospital*, 1939, **65**, 212.

⁶ Biskind, G. R., *Proc. Soc. Exp. Biol. and Med.*, 1940, **43**, 259.

Part of the graft tissue from each animal was preserved in Bouin's fluid.

With a few exceptions, the findings were fairly uniform. In almost all cases the implanted tissue appeared to be fairly healthy, seminiferous tubules were visible under the dissecting microscope. In the intra-mesenteric location, the grafted tissue was usually spread out over a considerable area, closely associated with the pancreas, so that the tubules in these grafts were somewhat more dispersed than in the subcutaneous grafts.

The animals with intra-mesenteric implants gave no evidence of androgenic stimulation. The penis was small, poorly developed and barely evertable; the prostates and seminal vesicles were small and atrophic in appearance. The animals with subcutaneous implants, however, showed some degree of androgenic stimulation in that the penis was generally normal in size and structure and the prostates and seminal vesicles were well above the castrate level in weight.

The weight findings in those animals having healthy implants are recorded in Table I. In the first set of experiments, the number of animals was small, therefore no attempt at statistical analysis was made. In the second set of experiments the prostate and seminal vesicle weights of the subcutaneous group were significantly larger than those of the intra-mesenteric group. The thymus weights were not significantly different.

The implants from all animals were sectioned and examined microscopically. In most cases the implanted tissue appeared to be well established and well vascularized. Regions of lymphocytic

TABLE I.
Prostate and Seminal Vesicle Weights in Rats with Testicular Implants.

Situation of implants	No. operated	No. survived to autopsy	No. with healthy implants	Avg body wt	Avg prost. wt	Avg S.V. wt	Avg thymus wt	σ
Operated at 22-23 days of age. Implants resident for 2 months.								
Intra-mesen.	10	7	5	213	4.92	5.93		
Subcut.	10	4	4	187	29.37	29.37		
Operated at 20 days of age. Implants resident for 1 month.								
Intra-mesen.	15	13	12	146	5.26	0.29 7.03	0.26 509.6	31.9
Subcut.	11	11	10	142	23.77	4.19 17.85	2.89 433.9	45.0

$$\sigma \text{ derived by the formula } \sqrt{\frac{\sum \delta^2}{n(n-2)}}$$

$$\text{Significant difference} > 3 \sqrt{(\sigma_1)^2 + (\sigma_2)^2}$$

infiltration and fibrosis were observed. The seminiferous tubules which were found in all healthy implants resembled those of experimental cryptorchid testes in that generally Sertoli cells alone were present, although occasionally a few spermatogonia were observed. Interstitial cells were present in all implants. The number of interstitial cells varied from a few scattered cells between the tubules to fairly large areas of densely packed cells. The latter condition may be regarded as true interstitial cell hypertrophy. Generally the grafts which were *in situ* for 2 months exhibited better development of interstitial cells than those which were only one month old.

One fact has been shown by these experiments. If viable testicular tissue is situated so that its venous drainage passes directly through the liver, there is no evidence of androgenic stimulation or maintenance in the accessory structures. If, however, the testicular tissue is located so that it is associated with the peripheral circulation, there is some androgenic activity registered in the accessories. The conclusion that the liver is responsible for the lack of androgenic effect in the case of the intra-mesenteric grafts is valid only if it can be shown that the graft tissue in this location is actually producing androgens. Jeffries⁷ and others have shown that testes rendered cryptorchid continue to produce androgens, in normal or nearly normal amounts, for a considerable period, even when the germinal elements are completely degenerate. In the grafted tissues from the animals reported here, interstitial cells were present and, in some cases, hypertrophied. This finding cannot be regarded as absolute proof of secretory function in the grafted tissue, but is strongly indicative of such function.

Summary. When testicular tissue was implanted into the immature rat so that its venous drainage passed through the liver, there was a lack of androgenic stimulation evident in the castrate condition of the penis, prostate and seminal vesicles after a period of one to 2 months. When the testicular tissue was implanted subcutaneously, some androgenic effect on the penis and accessories was obtained. Interstitial cells in varying numbers were present in all grafts. This finding indicates probable endocrine function by the implanted tissue. It is tentatively concluded that the lack of androgenic stimulation in animals with intra-mesenteric implants of testicular tissue is due to inactivation of testicular androgens by the liver. This is in agreement with similar experiments using implanted crystals of testosterone propionate and methyl testosterone.^{5,6}

⁷ Jeffries M. E., *Anat. Rec.*, 1931, **48**, 131.

11429

Antiserum for Renin.*

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In some earlier experiments on renin tachyphylaxis in the dog,¹ it was occasionally noticed that when an animal had become tolerant to dog renin it would still give a pressor response to the injection of rabbit renin. This observation, in the light of increasing evidence to support the protein-like character of renin, suggested the possibility of a species difference in this pressor substance as extracted from the renal cortex of these animals. Consequently, the idea occurred to us that a study of the immune responses to renin may furnish a new experimental approach to elucidate further its nature and properties. Even if renin is a hormone, as has been suggested, it may still produce recognizable immune or antihormone responses equally valuable for our immediate purpose.²

This report is concerned with the preparation and properties of an antiserum produced in rabbits injected with pressor active extracts of the renal cortex of the dog. A preliminary observation on the pressor negating effect of serum from a dog receiving injections of hog renin is also recorded.

The method used for the extraction of renin was essentially that described by Grossman.³ Dry, powdered, cortical residue, left after alcohol or acetone extraction, was used as crude material from which extracts of the pressor active principle were made. These (extracts) were further purified to remove much of the associated protein. About 30 cc of this partially purified extract was mixed with an equal volume of colloidal aluminum hydroxide. The colloidal aluminum hydroxide with the proteins, including renin, adsorbed on it was concentrated to half the volume by centrifuging and this gelatinous mixture injected intramuscularly into one rabbit.⁴ At the end of 2 or 3 weeks the rabbits were bled to ascertain the presence of antibodies in the serum. If antibodies in appreciable titer were present

* This work was aided by a grant from the Graduate School Research Fund of the University of Illinois.

1 Wakerlin, G. E., and Johnson, C. A., *Proc. Am. Phys. Soc.*, 1940, p. 192.

2 Collip, J. B., *Ann. Int. Med.*, 1934, **8**, 10.

3 Grossman, E. B., *Proc. Soc. Exp. Biol. and Med.*, 1938, **39**, 40.

4 Hektoen, L., and Welker, W. H., *J. Infect. Dis.*, 1933, **53**, 309.

the animal was bled to death and the antiserum preserved in the refrigerator. If a low titer serum was found several intravenous injections of the original extract were given to augment its titer. Immunization may also be accomplished by the more common method of injecting the soluble antigen (renin) intravenously in increasing amounts at 3-day intervals.

Since the antigen used was a mixture of renin, kidney protein(s), and serum proteins (especially pseudoglobulin), precipitins for the last were demonstrable in the antiserum. Precipitins for dog serum pseudoglobulin in the antiserum could be removed by *in vitro* adsorption without affecting the renin antibody. To demonstrate the presence of an antibody for renin we had to depend on the bioassay of mixtures of the antiserum and renin. Such assays were always controlled in the same animal by a similar dose of renin mixed with normal rabbit serum. After a number of attempts to determine the optimum quantity relationship between antiserum and renin we routinely mixed 2 volumes of antiserum with one volume of renin and allowed these mixtures to remain at 4°C at least overnight.

Dogs were used for all assays. The femoral artery was cannulated under light ether or local (procaine) anesthesia and the blood pressure recorded with a mercury manometer. Some of the dogs were also subjected to bilateral nephrectomy. After recovery from these operative procedures, the animal was given an initial dose of renin intravenously, usually equivalent to one-half gram of

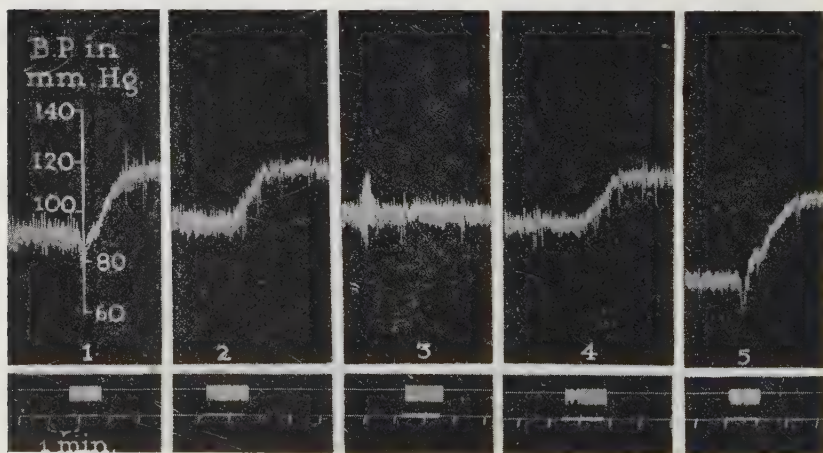


FIG. 1.

Dog, 7 kg, male, bilaterally nephrectomized. 1 Initial dose (2 cc) of dog renin injected, 2 two cc dog renin mixed with four cc normal rabbit serum, 3 two cc dog renin mixed with four cc antiserum for dog renin, 4 repeat of 2, 5 repeat of 1.

kidney per kilo of body weight, to establish the degree of pressor response. When the blood pressure had again assumed its original level a control dose of renin mixed with normal rabbit serum was injected. Figs. 1 and 2 illustrate results which are typical of those obtained by the assay of antiserum from 4 rabbits on a total of 15 dogs.

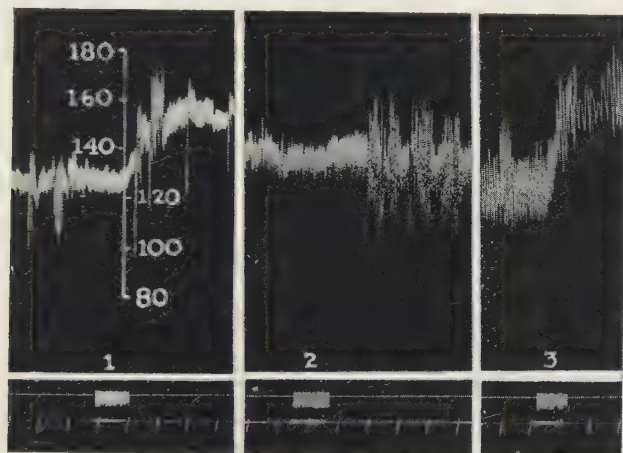


FIG. 2.

Dog, 8 kg, female, not nephrectomized. 1 One part (2 cc) dog renin mixed with two parts normal rabbit serum, 2 one part dog renin mixed with two parts antiserum for dog renin, 3 repeat of 1.

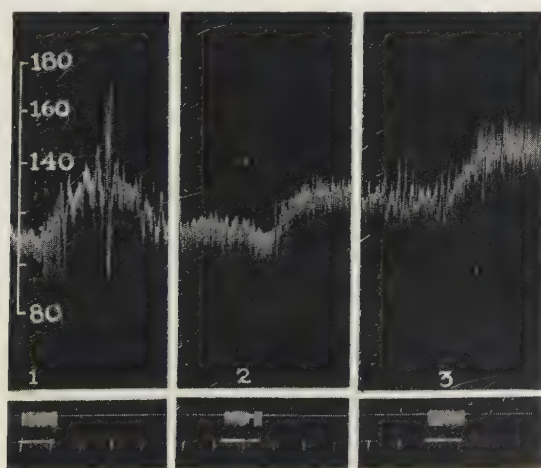


FIG. 3.

Dog, 20 kg, male, not nephrectomized. 1 One part (5 cc) rabbit renin mixed with two parts normal rabbit serum, 2 one part rabbit renin mixed with two parts antiserum for dog renin, 3 repeat of 1.

In 4 dogs we observed the effect of the injection of mixtures of rabbit renin and the antiserums for dog renin just described. The neutralizing effect of these antiserums on the pressor response to rabbit renin was distinctly less than it was for dog renin, as illustrated by Fig. 3. These results suggest a partial antigenic similarity between dog and rabbit renins although further work is necessary to prove this point conclusively.

Since pituitrin has a pressor effect similar to renin on the peripheral vascular bed it was of interest to determine whether the antiserum had any effect on the pressor response to pituitrin. Comparing injections 4 and 5 in Fig. 4, it is apparent that the mixing of pituitrin and antiserum did not change the character or degree of response to this agent as determined on 2 dogs.

In connection with another phase of our study of renin we had occasion to examine the serum of a dog which had received daily intramuscular injections of hog renin in a dosage of one gram of kidney equivalent per kilo for a period of 12 weeks. When serum from this dog was mixed with hog renin and injected into 3 assay dogs, a decidedly diminished response was observed (Fig. 5). No precipitins for hog serum proteins were demonstrable in this antiserum.

The neutralizing substance for renin demonstrated by these results may prove useful in throwing light on the question of whether renin is the pathogenetic agent in experimental renal ischemic hyper-

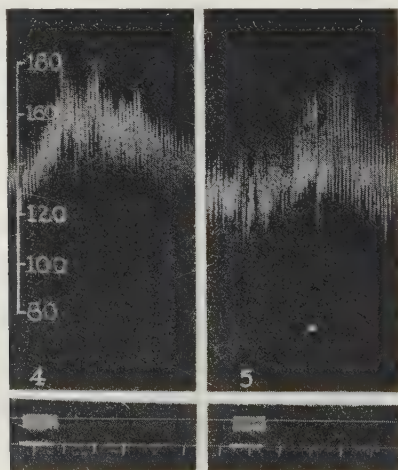


FIG. 4.

Dog, (same as in Fig. III.). 4 Three units pituitrin mixed with four cc antiserum for dog renin, 5 three units pituitrin mixed with four cc normal rabbit serum.

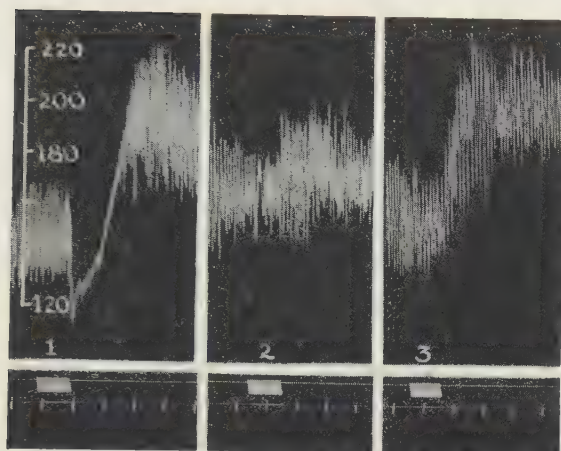


FIG. 5.

Dog, 7 kg, male, not nephrectomized. 1 One part (2 cc) hog renin mixed with two parts normal dog serum, 2 one part hog renin mixed with two parts serum from dog which had received injections of hog renin, 3 repeat 1.

tension. Speculating further, such an "antirenin" may conceivably have therapeutic value, when passively administered or actively produced in experimental renal ischemic hypertension. Experiments aimed at elucidating these possibilities, as well as a study of the immune responses to renins of other species, are now in progress.

Conclusions. 1. The rabbit is able to produce an antiserum which counteracts the pressor effect of dog renin. 2. The antiserum for dog renin appears to diminish the pressor response to rabbit renin. 3. This antiserum has no effect on the pressor action of pituitrin. 4. The dog appears able to produce an antiserum to hog renin. 5. The active principle of the antisera (antirenin) is most likely a non-precipitating antibody or possibly an antihormone. 6. Studies are now in progress to determine the value of antirenin in the therapy of experimental renal hypertension and also in the elucidation of the possible pathogenetic rôle of renin in this form of hypertension.

Tissue Culture Growth Stimulants from Ground Frozen-Dried Chick Embryos.

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It has been demonstrated¹ that frozen-dried plasma and similarly treated embryo juice used in combination, after solution in distilled water, form an adequate and satisfactory medium for the growth of tissue cultures. Further experimentation with such preparations has brought forth evidence that a much more potent embryo juice could be secured if the embryos themselves were ground and frozen-dried before extraction.

Eleven-day chick embryos were removed from their shells and membranes and reduced to a gray, grumous mass with sea sand in a Ten Broeck grinder. The material was pipetted off and allowed to stand in a container for a short while to permit the larger particles of grit to settle. Later the ground substance was introduced into pyrex ampoules, frozen-dried and sealed *in vacuo* by means of a Lyophile² apparatus. All manipulations were carried out aseptically.

The dried matter at first was crusty but could be reduced to a powder by forcibly shaking or by tapping the containers. The ampoules were stored at room temperature for 14 months before any tests were instituted. A preliminary series of cultures was made with embryo juice prepared by steeping a portion of the dried powder in Ringer-Tyrode's solution in an ice box prior to centrifuging. The cultures grew so exuberantly that it was decided to test statistically the growth-promoting effect of this extract on cultures of embryo chick heart.

By calculation from fresh embryos dried to constant weight in an oven, 70 mg of dry powder were equivalent to one gram of fresh embryo. This figure was only approximately accurate since there was no simple way of determining how much sand and glass dust might have been retained in the powder. However, 20% embryo juice was made by adding 70 mg of powder for each 5 cc of Ringer-Tyrode's solution. The mixture was agitated gently, steeped for

¹ Hetherington, Duncan C., and Craig, Jane Stanley, *PROC. SOC. EXP. BIOL. AND MED.*, 1939, **42**, 831.

² Flossdorf, Earl W., and Mudd, Stuart, *J. Immunol.*, 1935, **29**, 389.

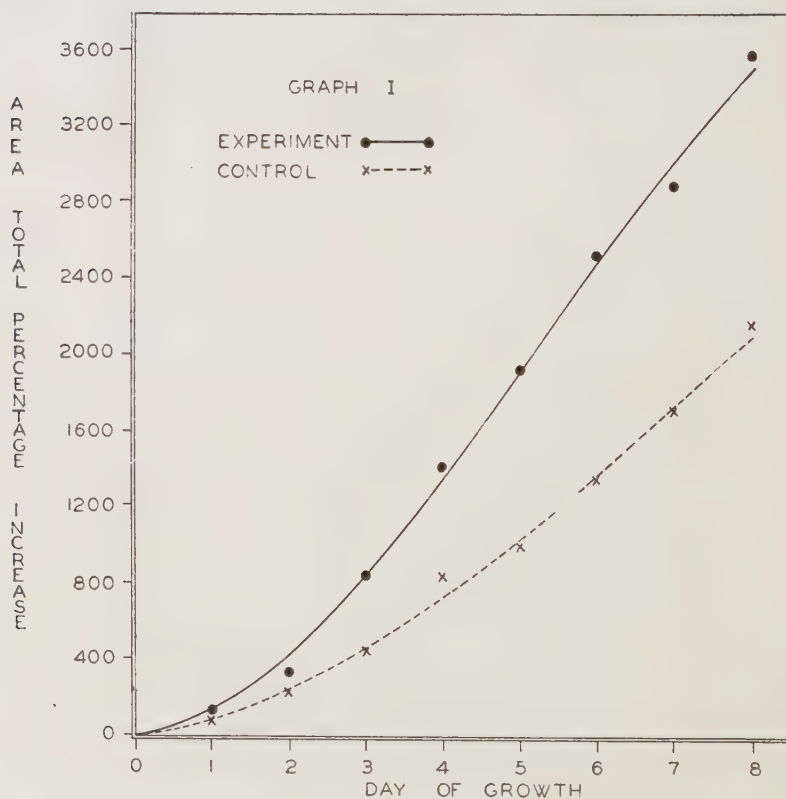
TABLE I.

Days of growth	Control Series				Experimental Series			
	Mean total area in mm ²	Probable error of mean	Total growth in %	D _M	Mean total area in mm ²	Probable error of mean	Total growth in %	σ
0	0.77	± 0.02	0	0.13	0.04	± 0.02	0	0.31
1	1.45	± 0.05	88	0.04	0.11	± 0.05	133	0.81
2	2.51	± 0.07	225	0.32	0.19	± 0.10	342	1.46
3	4.33	± 0.17	462	1.75	0.46	± 0.28	850	3.92
4	7.28	± 0.22	845	2.48	0.71	± 0.38	1425	5.47
5	8.61	± 0.32	1018	4.36	1.13	± 0.40	1926	5.74
6	11.26	± 0.38	1362	5.56	0.85	± 0.43	2528	6.08
7	14.06	± 0.39	1726	5.02	0.91	± 0.47	2881	6.53
8	17.54	± 0.40	2178	5.84	0.88	± 0.43	3553	6.14

D_M, difference of the means; σ_D , standard error of the difference of the means; significant difference = $D_M > 3\sigma_D$.

24 hours in an ice-box and centrifuged. The resultant slightly pink, opalescent fluid was removed and delivered in 1 cc quantities into ampoules and frozen-dried. Again aseptic technic was employed throughout. After 3 months storage this frozen-dried embryo juice made from the frozen-dried embryos was tested for growth-promoting properties. Two series of tissue cultures were planted from 11-day chick embryo hearts. At any one planting the same heart was used for both the experimental and the control cultures. The former were planted in equal parts of the new type embryo juice and frozen-dried plasma (stored for 6 months); while the latter were similarly treated except that 20% juice from fresh 11-day embryos was employed.

Delineascope and planimeter records were kept of the areas of the original explants; thereafter for 8 days, the total area of each culture was measured at 24-hour intervals. The results obtained from 90 cultures in each series of the experiment appear in Table I and Graph 1. From these it will be seen that there was a significant



growth increase in the experimental series, indicating rather conclusively the greater potency of the embryo-juice. Furthermore, careful microscopic examination of the living cells indicated that those in the test series did not develop fat droplets as readily as the controls and at all times appeared in better condition.

Fowler³ in a series of titrations of embryo juices upon chick heart fibroblasts concluded that best growth was obtained from utilizing extracts from 11- to 14-day whole embryos since during that growth period greater morphological and physiological changes took place within the developing animal—attributed possibly to endocrine production.

The potency of embryo juice prepared by the present method may be ascribed to increased accessibility of the cell contents to extraction and solution by the Ringer-Tyrode's saline. The preliminary grinding reduced the tissues to smaller particles than could be accomplished by mere mincing and the subsequent freezing* and drying disrupted most of the cells. In consequence many more substances from all parts of the embryo went into solution, probably in an undenatured condition.^{2, 4}

The experiment reported has shown that a very excellent embryo juice may be prepared from ground frozen-dried chick embryos. The dried powder retained its potency for 14 months and frozen-dried embryo juice derived from it was extremely active after 3 months' additional storage. There seems hence to be no reason why such products as frozen-dried embryo powder and frozen-dried plasma could not be used to considerable advantage in tissue culture laboratories.

³ Fowler, Ona M., *J. Exp. Zool.*, 1937, **76**, 235.

* Neutral red spreads made from tissues of chick embryos frozen to -70°C and then thawed rapidly at 37°C showed that very few of the cells remained intact. The nuclei alone appeared to be unbroken.

⁴ Elser, William J., Thomas, Ruth A., and Steffin, Gustav I., *J. Immunol.*, 1935, **28**, 433.

Behavior of Pigment Cells from Cultures of Neural Crest When Grafted Back into the Embryo.*

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Neural crest taken from chick embryos in early somite stages produces large numbers of pigment cells both *in vitro*,¹ and in grafts to the limb buds of older host embryos, the melanophores later appearing in the dermis and feathers of the hosts.² This work has been corroborated by Eastlick,³ who transplanted limb buds with or without the neural crest, and found that the appearance of pigment was correlated with the presence of the neural component in the grafts.

The earlier cultures were short time cover slip preparations designed to test the capacity for differentiation of the tissue explanted. In view of the complex prospective potency of the neural crest in the embryo, it seemed advisable to attempt to develop pure cell strains in order to study the behavior of these cells under controlled conditions. For the past year, explants of early somite neural crest from colored breeds have been grown in small culture flasks in a standard medium of plasma and embryonic extract, according to the technic developed by Carrel and his associates. Such cultures grow rapidly during the first week *in vitro*, soon producing a ring of dark brown or black cells. Growth then continues at a slower rate, and strains of cells are produced which are uniform in appearance and behavior, being composed of typical branched melanophores which spread out upon the clot or become aggregated in clumps.

Cells from these cultures have been grafted to embryos of another breed than that of the donor, a single culture furnishing enough tissue to graft to as many as 30 host embryos, without entirely exhausting the original strain. When a graft is inserted into the limb bud of a white host (which is subsequently observed through a transparent window in the shell), the ectoderm heals over the tissue,

* This research was supported by a grant from the Rockefeller Foundation to Yale University for work in experimental embryology under the direction of Professor Ross G. Harrison.

¹ Dorris, F., *Proc. Soc. Exp. Biol. and Med.*, 1936, **34**, 448; *Roux Arch.*, 1938, **138**, 323.

² Dorris, F., *Anat. Rec.*, 1938, **70**, 91; *J. Exp. Zool.*, 1939, **80**, 315.

³ Eastlick, H. L., *Collecting Net*, 1938, **13**, 151; *Genetics*, 1939, **24**, 98; *Anat. Rec.*, 1939 a., **73**, Suppl. 2, 64; *J. Exp. Zool.*, 1939 b., **82**, 131.

which is still clearly visible as a dark mass below the surface. During the first 2 days, migration of melanophores from the graft occurs, and these are finally found in the dermis, epidermis, and developing feathers of the femoral tract in host embryos fixed at the 12th to 15th day of incubation. A total of 145 grafts have been made, the tissue being furnished by pure strain cultures of differentiated melanophores obtained from the neural crest of Australorp embryos (a Black Orpington breed), the hosts being White Leghorn or Rhode Island Red embryos ranging in age from 3 to 5 days of incubation. The work, which is being extended to include other breeds of fowl, will be reported fully elsewhere.

Two points brought out by these experiments are of special interest. One is the maintenance in grafts, of the slow growth rate established *in vitro*. In striking contrast to the behavior of neural crest grafted directly from the embryo, which produces pigmented areas covering the whole leg, shank, and foot, as well as parts of the dorsal and ventral trunk regions, neural crest melanophores from cultures formed patches of pigment identical in appearance, but very much smaller in extent, never involving even the greater part of the femoral feather tract. Even more interesting is the fact that these cells, although transplanted into an environment composed of embryonic tissues physiologically younger, and thus forming what has been described as an "embryonic field", are apparently unaffected, and continue to reproduce as pigmented cells, behaving in a manner quite similar to that seen *in vitro*. This stability of both differentiation and growth rate in the formed tissue, is in striking contrast to the extreme lability of the embryonic region from which the cells were originally derived.

Experimental Tuberculosis in the Cotton Rat (*Sigmodon hispidus littoralis*).*

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Armstrong¹ was the first to discover that the Eastern cotton rat differs from other rodents in that it proved susceptible to the virus of poliomyelitis. Similar results were recently obtained by Jungeblut and Sanders² with another strain of monkey poliomyelitis virus. Since then there has been carried out in this department a systematic study of the reaction of cotton rats to other infections and toxic agents. It was found that this animal (*Sigmodon hispidus littoralis*) resembles the guinea pig in its susceptibility to diphtheritic toxin and bacillary infection with *C. diphtheriae*.³ This resemblance between the two species was also shown to exist with respect to their susceptibility to infection with *Trypanosoma equiperdum*.⁴ It seemed of interest to determine how this animal compares with the guinea pig and the albino rat in its susceptibility to infection with tubercle bacilli.

Experimental Methods. Seven albino rats (averaging 100 g in weight), 7 cotton rats (averaging 80 g), and 7 guinea pigs (averaging 350 g) were tested intracutaneously with 1 mg O.T. and found to be negative. All animals were then infected intravenously with 1 mg of bovine tubercle bacilli (B1). In some instances part of the inoculum escaped into the surrounding tissue as evidenced by the occurrence of infection of the regional glands. Two weeks after infection all animals in the 3 groups were again tested intracutaneously with 1 mg O.T. Half of the survivors in each group were sacrificed for pathological studies 46 days following infection; at the end of 2 months the remaining animals were tested for susceptibility to tuberculin-shock.

Results. Guinea Pigs: Six animals which survived longer than 2 weeks were found to be highly sensitive to intracutaneous doses of

* Aided by grants from the National Tuberculosis Association and the Philip Hanson Hiss, Jr., Memorial Fund.

¹ Armstrong, C., *Pub. Health Rep.*, 1939, **34**, 1719.

² Jungeblut, C. W., and Sanders, M., *Proc. Soc. Exp. Biol. and Med.*, 1940, **44**, in press.

³ Jungeblut, C. W., *Proc. Soc. Exp. Biol. and Med.*, 1940, **43**, 479.

⁴ Culbertson, J. T., *J. Parasit.*, in press.

1 mg O.T. All 7 died within 12 to 37 days after infection and showed extensive parenchymatous tuberculosis with caseation. It was interesting to note that 5 of these guinea pigs had miliary tuberculosis of the kidneys, a condition not encountered with subcutaneous or intraabdominal infection.

Albino Rats: All of these rats were negative to intracutaneous doses of 1 mg O.T., and tolerated 500 mg of O.T., injected subcutaneously, without noticeable symptoms. At necropsy, 46 days after infection, 3 of 4 animals showed a sparse scattering of tubercles in the lungs, and some suspicious areas in the spleen. No other macroscopic evidence of tuberculosis was found. The 3 remaining animals were sacrificed 65 days after infection, subsequent to unsuccessful attempts at shocking them with 1 g O.T. These rats showed the same scant tuberculous involvement as those sacrificed earlier.

Cotton Rats: All of the cotton rats reacted negatively to the intracutaneous injection of 1 mg O.T. 2 weeks after infection. All 4 animals which had been sacrificed at 46 days showed miliary tuberculosis of the lungs, spleen, and lymph glands. One cotton rat had miliary tuberculosis of the liver, and 3 showed tubercles in the kidneys. In all 4 cotton rats definite tissue-destruction and caseation was observed at the site of infection.

Of the 3 surviving animals, one died 65 days after infection, and showed extensive tuberculosis in the lungs, liver, and kidneys. The 2 remaining cotton rats were given 250 mg O.T. subcutaneously at the same time. One died the following day with no evidence of tuberculin shock. The other cotton rat showed no symptoms. It was then injected subcutaneously with 500 mg O.T. No evidence of tuberculin-intoxication was observed during the next 24 hours. The same animal was then injected intraabdominally with 1 g O.T. Within 5 minutes after injection, the animal became restless and dyspneic, and died within 40 minutes. At necropsy, there was serous fluid in the thoracic and abdominal cavities, and focal hemorrhages were found about the tubercles in the various organs. Both of the last 2 cotton rats showed miliary tuberculosis of the lungs, liver, spleen, glands, and kidneys.

Histopathology: Histological study of the tissues confirmed and further emphasized the differences found at autopsy. The tubercles in the cotton rat are predominantly epithelioid in type, some showing caseation, but no giant cells were observed. In most sections the tubercles were conglomerated. Acid-fast bacilli are easily demonstrated in the tubercles. A more complete description of the histopathology will be given in a later communication.

Summary and Conclusions. A comparative study of the Eastern

cotton rat, the albino rat, and the guinea pig suggests that the cotton rat occupies a position midway between the other two species regarding its susceptibility to infection with pathogenic bovine tubercle bacilli. It is definitely more susceptible than the naturally resistant albino rat but not as highly susceptible as the guinea pig. Like the albino rat, the tuberculous cotton rat is insensitive to skin-test doses of tuberculin, and tolerates large amounts injected subcutaneously. Since the observations on tuberculin shock are based upon findings obtained from only one animal, further work is necessary to confirm this point.

11433 P

Hypoaminoacidemia in Patients with Pneumococcal Pneumonia.

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The observations by Farr and MacFadyen¹ and Farr² on the incidence and duration of hypoaminoacidemia in children with the nephrotic syndrome have reopened the question of the constancy of blood amino acid concentration in other disease states. Because of certain clinical similarities between the onset of recovery in pneumococcal pneumonia and that of children from nephrotic crises, as well as the prevalence of pneumococcal infections in nephrotic children, we believed that extension of studies on blood amino acids to diseases other than Bright's disease might profitably begin with pneumonia.

Studies of plasma amino acids on all patients admitted to the pneumonia service in this hospital have been made. While these are not yet completed, the results thus far have been sufficiently striking and uniform to warrant reporting them.

Methods. Blood was drawn from each patient immediately upon admission to the hospital and before any therapy was begun. Subsequently, blood was drawn at selected intervals, when possible after an overnight fast, otherwise after an interval of at least 4 hours had elapsed from the time when the last protein-containing food was given. The blood was drawn with care to prevent hemolysis, ox-

¹ Farr, L. E., and MacFadyen, D. A., *Am. J. Dis. Child.*, 1940, **59**, 782.

² Farr, L. E., *J. Ped.*, in press.

alated, and immediately centrifuged. The plasma was pipetted off and the sample kept in the icebox until the analyses could be run. Plasma amino acid nitrogen was determined by the ninhydrin- CO_2 method of Van Slyke and Dillon³ as applied to blood by MacFadyen and Van Slyke.⁴ Farr and MacFadyen¹ have already pointed out that

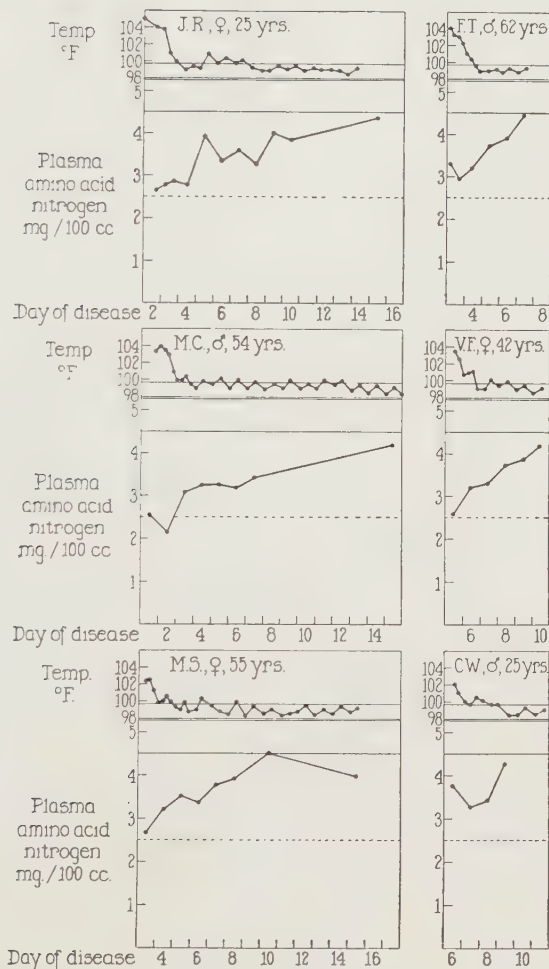


FIG. 1.

Graphic summary of results of serial plasma amino acid nitrogen determinations on 6 patients with pneumococcal pneumonia. The average normal value for plasma amino acid nitrogen is 4.50 mg per 100 cc. The critical value for nephrotic crises is 2.5 mg per 100 cc as indicated by the broken line. Note the pronounced hypo-aminoacidemia during the acute phase of the disease followed by a rise during recovery. Temperature charts are included to briefly summarize the clinical course.

³ Van Slyke, D. D., and Dillon, R. T., *Compt. rend. Lab. Carlsberg*, 1938, **22**, 480.

⁴ MacFadyen, D. A., and Van Slyke, D. D., in preparation.

even the Van Slyke nitrous acid method⁵ may be inadequate to permit detection and interpretation of small changes in plasma amino acid levels. For this reason the ninhydrin-CO₂ method was used in this study because of its greater specificity.

Results. The results on 6 patients are summarized graphically in Fig. 1. As early as the first day of disease a distinct drop in the plasma amino acid nitrogen had occurred. With recovery from the pneumonia all patients showed a rise of the plasma amino acid nitrogen to normal levels. Whereas the average concentration of plasma amino acid nitrogen in nephrotic children was about 3 mg % which decreased to as little as 1.2 mg % during nephrotic crises,^{1,6} the lowest value thus far observed in pneumonia patients was 2.17 mg %. Data at hand indicate the normal average plasma amino acid nitrogen to be 4.50 mg per 100 cc with the standard deviation ± 0.46 .

All patients in the present series were treated with sulfapyridine. Unpublished data indicate that in therapeutic doses this drug has no effect upon the plasma amino acid concentration.

Studies are at present under way in this clinic on the plasma amino acid nitrogen concentration in a variety of acute infectious diseases and in a few selected metabolic disorders. Until additional data have been obtained, the physiological significance of hypoaminoacidemia cannot profitably be discussed.

Summary. Observations on the plasma amino acid nitrogen of 6 patients with pneumococcal pneumonia are presented. In each instance during the acute phase of the disease the patient showed a plasma amino acid concentration significantly below the average normal value. During convalescence there was a gradual rise in the concentration of plasma amino acid nitrogen, with a return to a normal level on complete recovery from the disease. Present data indicate the normal average value to be 4.50 mg per 100 cc.

⁵ Van Slyke, D. D., *J. Biol. Chem.*, 1929, **83**, 425.

⁶ Farr, L. E., *Am. J. Dis. Child.*, 1939, **58**, 939.

11434

Squid Melanin: A Naturally Occurring Reversibly Oxidizable Pigment.*

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It was reported previously that synthetic melanin resulting from either the enzymatic oxidation of tyrosine or auto-oxidation of dihydroxyphenylalanine was reversibly oxidizable.¹ It was also found that natural melanin was reversibly oxidizable but that the reaction was far more sluggish and barely perceptible. This was thought to be due to the necessarily low concentrations obtainable and the contaminations present in the solutions of natural melanin.¹

The change in percentage light absorption on reduction of synthetic melanin was very marked. It was thought that these could be duplicated if a source of relatively pure and concentrated natural melanin could be found. Such a source was found in the so-called ink of the ink-sac of the squid (*Loligo pealii*). The ink is a highly concentrated, relatively pure colloidal solution of melanin. The particles were so small that they were invisible under the ordinary microscope, but visible with dark field illumination.

The ink was collected by removing the squid from the water with a net as carefully as possible to prevent discharge of the ink. The mantle was slit immediately with a pair of long scissors. The ink-sac was then dissected out without much danger of being discharged. Many attempts to anesthetize or immobilize the animal by cooling almost always caused the discharge of most of the ink. The ink was withdrawn with a syringe or expressed into a vial after the sphincter had been removed. The ink collected in this manner was extremely concentrated. After diluting with 300 volumes of water, an ink solution, approximately 1 cc in thickness, would transmit an amount of light that was barely registered by a sensitive photoelectric colorimeter.

For purposes of comparison, a solution of dopa melanin was prepared by auto-oxidation in air. The concentrations of the squid melanin and the dopa melanin solution were adjusted so that they

* Aided by a grant from the American Association for the Advancement of Science.

¹ Figge, F. H. J., *PROC. SOC. EXP. BIOL. AND MED.*, 1939, **41**, 127.

both absorbed 90% of the light. Both solutions were then reduced with sodium hydrosulphite and the light absorption values again determined. The reduced dopa melanin absorbed 66% of the light, while the reduced squid melanin absorbed 76% of the light. The melanin solutions were then re-oxidized with potassium ferricyanide. The re-oxidized dopa melanin absorbed 89% of the light, while the re-oxidized squid melanin absorbed 88.5%. This experiment was repeated 22 times with ink from 35 squid. The results were always uniform.

It may be seen from the light absorption values that the reversibility of the oxidation of the natural squid melanin approached that of synthetic dopa melanin. The figures indicate that the squid melanin was contaminated with some substance that absorbed about 10% of the light and which was not reversibly oxidizable. This substance may have been melanin that had aged in the squid ink-sac because even synthetic melanin 6 months old was not reversibly oxidizable.

11435 P

Relationship between "Spreading Factor" and Hyaluronidase.*

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Duran-Reynals¹ demonstrated the presence of an extractable factor in certain tissues and bacteria which enhances the invasiveness of some pathogenic agents. This factor has been extensively studied by Duran-Reynals and by others.^{2,3} Chain and Duthie⁴ reported that testis extracts containing "spreading factor" decrease the viscosities of synovial fluid and vitreous humor with the liberation of reducing substances. They suggested that "spreading factor"

* This work has been supported in part by a grant from the John and Mary Markle Foundation.

¹ Duran-Reynals, F., *Compt. rend. Soc. biol.*, 1928, **99**, 6; *J. Exp. Med.*, 1929, **50**, 327; *ibid.*, 1933, **58**, 161; *Yale J. Biol. and Med.*, 1939, **11**, 601.

² McClean, D., *J. Path. and Bact.*, 1930, **33**, 1045; *ibid.*, 1936, **42**, 477.

³ Claude, A., *J. Exp. Med.*, 1937, **66**, 353; Claude, A., and Duran-Reynals, F., *J. Exp. Med.*, 1937, **65**, 661.

⁴ Chain, E., and Duthie, E. S., *Nature*, 1939, **144**, 977.

is probably identical with the "mucinase" which hydrolyzes the polysaccharide in these fluids.

A mucopolysaccharide designated as hyaluronic acid has been isolated from vitreous humor, umbilical cord,⁵ the mucoid phase of Group A hemolytic streptococci,⁶ synovial fluid,⁷ fowl sarcoma⁸ and the pleural fluid of a patient with a mesothelioma.⁹ Enzymes which hydrolyze hyaluronic acid have been prepared from pneumococci, Group A hemolytic streptococci, *Cl. welchii*, and splenic tissue.¹⁰ More recently Meyer and Chaffee¹¹ confirmed the observation of Chain and Duthie and demonstrated that testis extracts hydrolyze isolated hyaluronic acid as well as the polysaccharide acid of cornea. The presence of hyaluronidase in high concentration has also been demonstrated in leech extract.¹²

The present study was undertaken to determine more precisely the relationship between "spreading factor" and hyaluronidase. The spreading capacity of the various preparations was tested by the intracutaneous method (using T.1824).¹ The action of hyaluronidase was tested (1) by the hydrolysis of isolated hyaluronic acid and (2) by determining changes in the viscosity of various fluids known to contain hyaluronic acid.†

The presence of hyaluronidase and "spreading factor" was tested in preparations from the following sources: pneumococci (Type I virulent and avirulent strains, Type II avirulent strain), Group A hemolytic streptococci (virulent and avirulent strains in the mucoid and rough phases), *Cl. welchii*, testis, pigskin, leech extract, commercial hirudine, and certain chemical substances.

Results. All preparations containing hyaluronidase were found to possess spreading properties. In addition hyaluronidase and "spreading factor" were found to possess certain attributes in common. The activity of both was weakened by heating at 65°C for

⁵ Meyer, K., and Palmer, J. W., *J. Biol. Chem.*, 1936, **114**, 689.

⁶ Kendall, F. E., Heidelberger, M., and Dawson, M. H., *J. Biol. Chem.*, 1937, **118**, 61.

⁷ Meyer, K., Smyth, E. M., and Dawson, M. H., *J. Biol. Chem.*, 1939, **128**, 319.

⁸ Kabat, E. A., *J. Biol. Chem.*, 1939, **130**, 143.

⁹ Meyer, K., and Chaffee, E., *J. Biol. Chem.*, 1940, **133**, 83.

¹⁰ Meyer, K., Hobby, G. L., Chaffee, E., and Dawson, M. H., *J. Exp. Med.*, 1940, **71**, 137.

¹¹ Meyer, K., and Chaffee, E., *PROC. SOC. EXP. BIOL. AND MED.*, 1940, **43**, 487.

¹² Claude, A., *PROC. SOC. EXP. BIOL. AND MED.*, 1940, **43**, 684. Unpublished data.

† The assumption has been made by some investigators that capacity to reduce viscosity constitutes a test for the presence of "mucinase." While it is apparently true that hydrolysis and decrease in viscosity are catalyzed by the same agents, it is not certain that the two reactions are due to the same enzyme.

30 minutes and destroyed at 100°C; both were destroyed or markedly weakened by iodine and, under the experimental conditions, neither was reactivated by sodium sulfite.

The theory that spreading factor and hyaluronidase are identical postulates the existence in skin of either hyaluronic acid or a similar substrate on which hyaluronidase may act. Recently, in this laboratory, a polysaccharide acid which is hydrolyzed by hyaluronidase preparations has been isolated from skin. The nature of this polysaccharide will be described elsewhere.

The evidence so far presented suggests that spreading factor may owe its action to the presence of hyaluronidase. On the other hand, certain observations suggest that the two are not identical.

A number of preparations which possessed marked spreading action did not hydrolyze hyaluronic acid nor did they reduce the viscosity of solutions containing the polysaccharide. Among these were several preparations from different strains of Group A hemolytic streptococci, pigskin, commercial hirudine, arsenious oxide and hyaluronic acid.‡

A further possible point of distinction was observed between hyaluronidase and "spreading factor." Antiserum made against hyaluronidase prepared from pneumococci specifically and completely inhibited the activity of the homologous enzyme but did not inhibit the spreading action of the pneumococcal preparations. This difference may be due to the combination of pneumococcal hyaluronidase with the antiserum to form a loose complex which may be inactive *in vitro* but may dissociate to an active form *in vivo*. It should be mentioned, however, that the antiserum to the pneumococcal enzyme did not inhibit the action of hyaluronidase prepared from streptococci nor did it affect the spreading action of such preparations.

Summary. Evidence is presented to show that hyaluronidase and "spreading factor" exhibit certain attributes in common. However, there is also considerable evidence that "spreading factor" does not owe its activity solely to the presence of hyaluronidase. It would seem probable that the phenomenon of "spreading" is a complex one and that several factors may be involved in its production. Further work is required to explain the mechanism of "spreading" in terms of known chemical and physico-chemical reactions.

‡ Duran-Reynals¹ has also shown that skin contains spreading factor. The spreading effect produced by simple chemical substances may be due to the release of "spreading factor" locally at the site of injection.

11436 P

The Sympathetic Component of the Sciatic Nerve.

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A. J. Goldforb.)

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College of Medicine.*

Our present knowledge of the course of the preganglionic nerve fibers is based largely on the early experiments of Langley^{1,2} and Bayliss and Bradford³ who used erection of hairs, sweating and vascular changes for recording sympathetic activity. These types of effector activity do not lend themselves readily to quantitative estimation. Moreover sweating and vasomotor changes offer additional difficulties in that they are complex responses. Sweating is influenced by accompanying vascular changes while vasomotor effects in turn are subject to rapid modification by compensatory reflexes.

In the present experiments a direct insight into the kind and magnitude of the sympathetic nerve impulses themselves was obtained by recording the action potentials in the efferent nerves. A direct current amplifier, which Marrazzi⁴ had found, in recording action potentials from other parts of the sympathetic system, to be especially suitable, was utilized to actuate a Matthews oscillograph. This technique provides not only a means of tracing the pathway of preganglionic fibers to an organ or a limb but enables the analysis of an exact point-to-point representation of the sympathetic component of each ventral root in any peripheral nerve.

Method. In a series of experiments on 19 cats, lightly anesthetized with nembutal or sodium amytal the spinal nerve roots from T7 to L7, inclusive, were severed from the cord, the dorsal root and ganglion excised, and the distal cut end of the ventral root stimulated, after insulation from surrounding tissues, by accurately controlled shocks from a thyatron stimulator. The activity resulting in the sciatic nerve was detected by electrodes placed on the main branches of the ipsilateral nerve and connected to the amplifier. The B and C waves thus recorded enabled us to map the exact roots

¹ Langley, J. N., *J. Physiol.*, 1891, **12**, 347.

² Langley, J. N., *J. Physiol.*, 1894-95, **17**, 296.

³ Bayliss, W. M., and Bradford, J. R., *J. Physiol.*, 1894, **16**, 10.

⁴ Marrazzi, A. S., *J. Pharm. and Exp. Therap.*, 1939, **65**, 18.

through which sympathetic fibers passed to the lower limb via the sciatic nerve. Since all slowly conducting fibers are not exclusively autonomic, the possibility that some of the B and C waves might have originated elsewhere was considered and excluded by the fact that the intravenous injection of nicotine, which in the doses given acts at autonomic ganglia without affecting postganglionic nerve trunks, was effective in blocking the previously recorded impulses.

Usually 3 or 4 roots were stimulated in each experiment. By separation of the medial and lateral popliteal nerves and recording from each in turn, while repeating the stimulus to the ventral roots, the sympathetic component of each division of the sciatic nerve was determined. Similarly, by recording from the contralateral sciatic, data were obtained on the extent of extraspinal crossed pathways between the sympathetic chains.

Results. Nicotine injected intravenously in doses producing a ganglionic block abolished both the B and C waves appearing after stimulating L1, L2 and L3. Nicotine has not yet been utilized in the experiments in which the other roots have been stimulated. The results show that in the cat the preganglionic outflow to the lower limb via the sciatic nerve emerges constantly from the spinal cord by the ventral roots as high as T11 and as low as L4, and that the roots giving the greatest contribution are T13-L3 (inclusive) of which L1 and L2 invariably gave a response. T11 rarely contributes and T12 and L4 only occasionally ($\frac{1}{4}$ - $\frac{1}{3}$ of the animals).

From the positive roots B and C waves or C waves alone were recorded. The C waves appeared in all cases except in 2 where the animal was in poor condition. In these apparently only the fibers of lower threshold responded giving B waves alone. Similarly, in several experiments, not tabulated because the records were taken immediately after cessation of the circulation, again B waves only were obtained.

Recording from the medial and lateral popliteal divisions of the sciatic nerve separately gave the same result as recording from the combined nerve. No responses from the contralateral sciatic nerve were ever obtained, if care was taken to avoid spread of current. Crossed pathways, between the sympathetic chains, of fibers destined for the opposite sciatic nerve have therefore not been demonstrated.

The results agree with Langley's^{1,2} analysis of outflow of *secretory* fibers to the hind limb of the cat, made from naked-eye observations of sweating on the foot pads during ventral root stimulation. In dogs Bayliss and Bradford³ used the plethysmographic method of

recording changes in volume in the lower limb during spinal root stimulation, and found *vasoconstrictor* fibers to the lower extremity emerging in T11-L2 (inclusive) and to a lesser extent in L3. Oughterson, Harvey and Richter⁵ indicate by recording the temperature changes in the lower limb of dogs after interrupting the vasomotor pathways by transection of the spinal cord, that vasoconstrictor fibers to the lower extremity may emerge below L6. Derom⁶ would limit the vasomotor fibers to the first 3 lumbar nerves in the dog, on the grounds that section of the rami communicantes of these roots abolishes all vasomotor reflexes in the lower extremity.

The existence of B as well as C waves, amongst the responses that were shown to be autonomic by their disappearance after nicotine, is of considerable interest. The presence of B fibers in the sympathetic outflow to the limb has not hitherto been clearly indicated, though Erlanger⁷ speaks of some "inconstant results" in cats and dogs. Such fibers imply the possibility of control over effectors differing in function and distinct from those supplied by fibers of the C group. The number of B and C fibers contributed by each root would then determine the possible extent of its control over the various types of sympathetic response.

11437

A Difference in Effect of Distilled Water and of Isotonic Solutions in Intestine on Pancreatic Secretion.

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The fact that water in the intestine stimulates the external secretory function of the pancreas was first demonstrated by Damaskin in Pavlov's laboratory^{1, 2} and later by Babkin^{3, 4} and Bylina.⁵ In the

⁵ Oughterson, A. W., Harvey, S. C., and Richter, H. G., *J. Clin. Invest.*, 1932, **11**, 1065.

⁶ Derom, E., *Mém. de l'Acad. Roy. de Méd. de Belg.*, 1938, **25**, 1.

⁷ Erlanger, J., in Erlanger, J., and Gasser, H. S., *Electrical Signs of Nervous Activity*, Philadelphia, University of Pennsylvania Press, 1937, p. 67.

¹ Pavlov, I. P., *Die Arbeit der Verdauungsdrüsen*, Weisbaden, 1898.

² Pavlov, I. P., *The Work of the Digestive Glands*, London, 1910, p. 144.

³ Babkin, B. P., *Arch. d. Sci. Biol.*, 1904, **11**, No. 3 (Reference from Babkin⁴).

⁴ Babkin, B. P., *Die äussere Sekretion der Verdauungsdrüsen*, Berlin, 1914.

⁵ Bylina, A. S., *Prakt. Arzt*, (russ) 1911, No. 44-49 (Reference from Babkin⁴).

course of an investigation of the effects on pancreatic secretion of various organic buffer mixtures in the intestine (results to be published elsewhere), we found that many neutral, isotonic, watery solutions were ineffective. The reason for this apparent conflict in results provides the material for the present report.

The dogs used in these experiments were provided with gastric and duodenal fistulas fitted with large cannulas ($\frac{5}{8}$ " lumen). The duo-

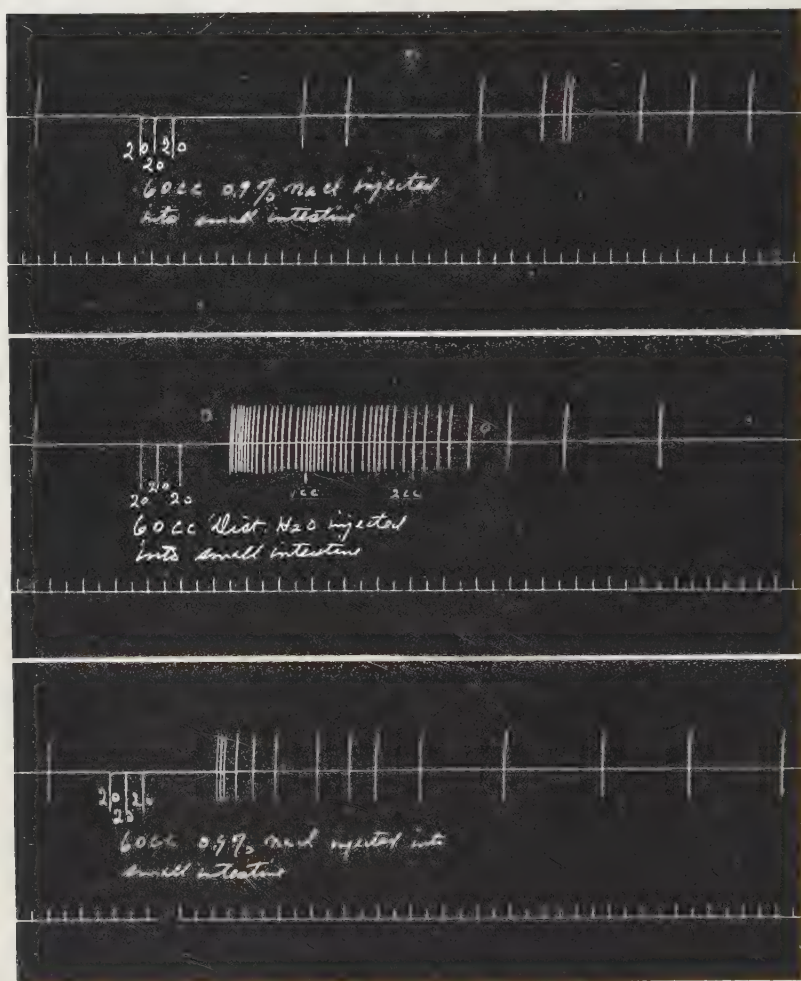


FIG. 1.

Effect of injecting 60 cc of distilled water (middle record) into the intestine compared with the effect of an equal amount of 0.9% NaCl solution (upper and lower records) given 25 min before and 25 min after the water. A drop recorder was used which delivered 17 drops per cc. Time is in 30 sec intervals.

denal fistula was placed opposite the point of entrance of the main pancreatic duct into the duodenum. The pancreatic juice was collected by means of a funnel shaped rubber cup, which was held against the duodenal wall surrounding the papilla by means of a suitable spring device. Further details of the method will be given in the report referred to above.

Water and various solutions were either injected into the upper small intestine in 20, 40, or 60 cc amounts through a tube passed via the duodenal cannula or perfused through the intestinal lumen by means of a pump delivering approximately 15 cc per minute.

Distilled water injected into or perfused through the intestine caused a brief but rapid flow of pancreatic juice. Various isotonic solutions, *e.g.*, 0.9% NaCl and 5% glucose had no such effect, except when given after an injection of distilled water, when a doubtfully positive result was frequently observed (Fig. 1).

Evidently the pancreatic secretagogue action of water is in some way related to hypotonicity and is not exhibited by the water present in isotonic solutions. This fact has an important bearing on the interpretation of experimental data obtained with watery solutions. Instead of concluding, as at present, that a substance in solution is inert if it has the same effect on the pancreas as an equal amount of pure water, we must regard it as an active stimulus if it is effective in isotonic solution.

These observations do not necessarily apply to water or solutions that have passed through the stomach where they may become mixed with HCl or, possibly, other pancreatic secretagogues.

Conclusion. Although pure water in the intestine causes pancreatic secretion, isotonic solutions of inert substances in water do not.

Ovarian Transplantations in the House Mouse.

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There have been many attempts to obtain offspring from homo-plastic transplants of ovaries in vertebrates. Of these attempts, the most important are those of Magnus¹ on rabbits, Guthrie² on hens, and Castle and Phillips³⁻⁵ on guinea pigs and rabbits. The results of Magnus and Guthrie are uncertain, however, due to the possibility of the regeneration^{6,7} of host ovarian tissue. Castle and Phillips grafted ovaries into 141 guinea pigs which differed from the donors in a single genetic character, and, of these, 3 produced young having the genetic characters of the donor animals. The present work deals with the results obtained from ovarian transplantations in the house mouse.

Three groups of experiments were carried out on mice 6-8 weeks old. These are: Group I. A single ovary was transplanted from an animal of one inbred line into an animal of a different inbred line. Group II. A single ovary was transplanted from an animal of an inbred line into an animal differing in a single genetic character but belonging to the same inbred strain as the donor animal. Group III. As in Group II but in which the remaining host ovary was completely removed.

The transplantations were carried out as follows: The ovary of the host animal was exposed by a dorso-lateral incision in the abdominal body wall. A small incision was made in the ovarian capsule as far distant from the Fallopian tube as possible, and the ovary removed. The donor ovary was then pushed through the slit into the host ovarian capsule. No sutures were needed except for closing the incision in the abdominal wall.

Group I. Ovaries from yellow and agouti mice of an inbred line were grafted into 36 female albino mice of 2 different inbred lines.

¹ Magnus, V., *Norsk Mag. for Laegevidensk.*, 1907, 5R, **5**, 1057.

² Guthrie, C. C., *J. Exp. Zool.*, 1907-08, **5**, 563.

³ Castle, W. E., and Phillips, J. C., *Science*, N. S., 1909, **30**, 312.

⁴ Castle, W. E., and Phillips, J. C., Carnegie Institution of Washington, 1911, Publ. No. 144.

⁵ Castle, W. E., and Phillips, J. C., *Science*, N. S., 1913, **38**, 783.

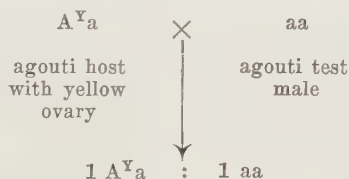
⁶ Davenport, C. B., *J. Exp. Zool.*, 1925, **42**, 1.

⁷ Haterius, H. O., *Physiol. Zool.*, 1928, **1**, 45.

The host albino females were test mated with albino males and all of the offspring obtained were albino, indicating that none of the ovarian grafts were successful. This is in agreement with the work on tissue transplantation by Loeb⁸ and Loeb and Wright⁹ on normal tissues of guinea pigs, and by Loeb and King¹⁰ on normal tissues of rats. They found that tissues transplanted between 2 different inbred lines almost invariably degenerate, and few, if any, successful grafts are obtained.

Group II. Ovaries from yellow mice were transplanted into agouti litter mates in 11 cases. The yellow allele is dominant to the recessive agouti allele and there should be some yellow offspring resulting from the mating of the host agouti females to agouti males if the ovarian grafts are successful. However, all of the 30 young obtained in the resulting 5 litters were agouti, showing that the grafts were again unsuccessful.

Group III. Successful ovarian grafts were obtained, however, when one agouti host ovary was completely removed, and, a week to 10 days later, an ovary from a yellow litter mate was grafted into the opposite side of the agouti host. Thus, the agouti host contained one ovary from a yellow litter mate and neither of its own ovaries. Eight such operations have been completed. Of these 8 agouti females 4 have given no litters (indicating either sterility or failure of the graft to take), and four have given a total of 9 yellow and 9 agouti offspring after matings with agouti males. These transplantations were successful, and the young produced are according to genetic expectation. Thus:



In second matings, in which the 4 agouti females containing the successful ovarian grafts were mated with yellow males (for genetic purposes), 3 of the 4 animals have given a total of 8 yellow and 3 agouti offspring. This also is according to genetic expectation, showing that the transplanted ovaries have continued to function. In all of the litter-bearing females in this group the ovarian graft was

⁸ Loeb, L., *Physiol. Rev.*, 1930, **10**, 547.

⁹ Loeb, L., and Wright, S., *Am. J. Pathol.*, 1927, **3**, 251.

¹⁰ Loeb, L., and King, H. D., *Am. Nat.*, 1935, **69**, 5.

successful, and the remainder of the experimental females gave no litters. This shows that there was no regeneration of functional host ovarian tissue. In the animals of Group II, however, the grafts were unsuccessful in all 5 of the females which gave litters. This, according to the results in Group III, is not due to regeneration of host ovarian tissue at the site of the operation, but is probably due to a physiological block between the host ovary of one side and the grafted ovary, since such a high percentage of successful grafts are obtained when the remaining ovary is completely removed. Thus, it seems evident that the presence in the host of one of its own ovaries changes some reaction which is necessary for graft maintenance.

Since the host agouti females of Group III were first mated approximately 10-15 days after the operations, and since 3 have given second litters, it is quite certain that the ovulations in the successfully grafted ovaries were not due simply to the mechanical stimulation of the operation. Additional experiments of this type are being done at the present time, and it is hoped that the high percentage of successful ovarian grafts can be equalled or increased. This type of operation should be of importance as an aid to the solution of problems in developmental genetics of mammals.

11439 P

Effect of Anterior Chordotomy on Essential Hypertension.

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Within the past 10 years there have been many reports concerning the surgical treatment of essential hypertension.^{1, 2} The methods used both in experimental animals and in man may be classified into 4 different groups:³ (1) Interrupting sympathetic outflow by cutting anterior nerve roots from T₆ to L₂,⁴ (2) dividing the splanchnic and the sympathetic chain above the diaphragm,⁵ (3) dividing the

¹ Braden, S., and Kahn, E. A., *Yale J. Biol. Med.*, 1939, **11**, 449.

² Craig, W. M., *Surgery*, 1938, **4**, 502.

³ Ascroft, P. B., *Lancet*, 1939, **2**, 113.

⁴ Page, I. H., and Heuer, G. J., *Am. J. Med. Sc.*, 1937, **193**, 820.

⁵ Freyberg, R. H., and Peet, M. M., *J. Clin. Invest.*, 1937, **16**, 49.

splanchnic nerves and the lumbar sympathetic chain below the diaphragm,⁶ and (4) removing the coeliac ganglion.⁷

These various methods, however, have proved to be only partially successful, and this has stimulated us to attempt a new approach to the surgical treatment of essential hypertension. Goldblatt's⁸ ingenious experiments on dogs have shown that total sympathectomy of the thorax and abdomen, and even pithing, have had no effects on the type of experimental hypertension he produced. Based on the consideration that various forms of sympathectomy may reduce the blood pressure of patients with essential hypertension, but that these results were often disappointing, it was at first the intention of one of us (O.H.) to effect a more complete sympathectomy by partially sectioning the autonomic tracts in the cord. We observed, also, that when routine anterior chordotomy was performed for reasons other than hypertension, the operation was usually followed by a prolonged lowering of the blood pressure. Later it was found that, as far as the arterial pressure was concerned, a maximum result could be attained by beginning the section one to two mm anterior to the dentate ligament and carrying it to the anterior median fissure, thus interrupting the anterior and anterolateral columns of the cord. Bilateral sections are made because unilateral sections were found to have little effect on the arterial pressure. It has been found that the optimal location for the section is at the 8th cervical segment.

In women with essential hypertension, chordotomy has been almost uniformly followed by a reduction of blood pressure to the normal figure for 4 to 6 months, after which it tends to return to a higher level, but only occasionally to the preoperative level. Moreover, this operation has not necessarily produced a disturbance in sweating. Most of the subjects on whom this operation has been performed have had advanced hypertension, with subjective symptoms, and all of them have been greatly benefited. Our results in the few male patients so treated have not been as successful as the females. The few male patients whom we have subjected to this operation had more advanced changes in the vascular tree than the females so that the difference in sex was probably not the determining factor.

The authors feel that a dual mechanism (central neurogenic, and peripheral) is probably involved in essential hypertension, and that chordotomy possibly eliminates the central neurogenic factor. Work is being carried out with the idea of proving or disproving this

⁶ Allen, E. V., and Adson, A. W., *Am. Heart J.*, 1937, **14**, 415.

⁷ Crile, G., *Ann. Surg.*, 1938, **107**, 909.

⁸ Goldblatt, H., *Surgery*, 1938, **4**, 483.

hypothesis, and, if it should be correct, then eliminating the central factor would leave only the peripheral (hormone?) mechanism which may become more amenable to drug therapy.

Some physiological effects of anterior chordotomy have been discussed.⁹ The result of chordotomy on motor function in some 90 cases will be published in the near future.

11440 P

Occurrence of Strains of Pneumococci Which React With More Than One Type-Specific Antipneumococcal Serum.

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Pneumococci are commonly classed among the best examples of bacterial type-specificity. While cross-reactions frequently occur involving types 3 and 8 and less frequently other types, they have usually been considered from the standpoint of the cross-reacting antibodies that sometimes develop during immunization rather than from the standpoint of the antigenic type-multiplicity which causes them.^{1, 2}

Several strains of pneumococci that conspicuously violate the prevailing conception of type-specificity have been isolated recently in this laboratory. Neufeld tests with sera of 3 different manufacturers show that each of these strains reacts with at least 3 type-specific sera.

Table I summarizes the reactions of 6 such strains. It will be noted that each strain shows somewhat greater capsular swelling with one serum (either type 29 or type 24) than with others. However, reaction is only slightly less with a second, and in some strains with a third, serum.

With the exception of the Hoge strain all 6 were isolated from 488 routine specimens submitted to this laboratory for typing between February 16 and April 3, 1940. Fifty-nine of these specimens were reported as containing more than one type of pneumococci, and 18 of them examined for the possible presence of multiple-react-

⁹ Hyndman, O., and Van Epps, C., *Arch. Surg.*, 1939, **38**, 1036.

¹ Lyall, H. W., and Odell, H. R., *Am. J. Hyg.*, 1939, **29** (Sect. B), 103.

² Noble, A., and Cameron, B. C., *J. Lab. and Clin. Med.*, 1939, **24**, 1.

TABLE I.

Pneumococcal strain	Reactions with type-specific sera		
	Maximal capsular swelling and massive agglutination	Slightly sub-maximal swelling and moderate to massive agglutination	Little or no capsular swelling and moderate agglutination
Hoge*	29	10	20
Weingart	29	20, 31	—
Thorpe	29	10, 20	—
Hinman	24	7	20
Brown	24	7	20
Walker	29	10, 20	—

* Englewood Hospital Laboratory, Chicago, kindly furnished the specimen from which this strain was obtained.

ing strains. From these the last 5 listed in Table I were recovered. There is reason for believing that such strains were present in 2 other specimens but escaped isolation.

To ascertain that a mixture of types was not responsible for the multiple-type reactions, each of the 6 strains was plated from young blood-broth cultures. From each plate well-segregated colonies were picked, each colony to a fresh tube of blood broth. This procedure was repeated not less than four times with each strain. No change in the reactions of any of the strains was produced by this treatment.

Table II shows the reactions of 4 strains with the sera of 3 principal producers of typing sera. For purposes of comparison and control one strain each of types 10, 20, 29, and 31 also was tested. These were obtained from one of the producers whose sera were included in the study (Manufacturer C). Young cultures in Felton broth, formalinized (1.0% commercial formalin) to inhibit autolysis, or saline suspensions prepared therefrom, were employed in the tests. The saline suspensions were prepared by centrifugalizing the cultures and resuspending the sediment in formalinized (1.0%) physiological saline, the final turbidity approximating that of tube number 8 of a Macfarland nephelometer. One 2 mm loopful of bacterial suspension, one 2 mm loopful of Loeffler's methylene blue and one 4 mm loopful of serum were mixed in each preparation examined under the microscope.

It is apparent from the second table that although the sera from the 3 sources differ somewhat in the scope and degree of their cross-reactions they severally substantiate the fact of multiple-type antigenicity in the strains tested. Even the control strains, types 10 and 20, do not appear to be perfectly specific. However, the reactions of

TABLE II.
Reactions Between Sera of 3 Manufacturers and 4 Multiple-reacting Strains of Pneumococci.

Cultures	Type-Specific Sera											
	Manufacturer A				Manufacturer B				Manufacturer C			
	10	20	29	31	10	20	29	31	10	20	29	31
Multiple-reacting strains												
Hoge	++	++	++	SLAg.	+	Ag.	++	SLAg.	++	++	++	—
Weingart	±	++	++	++	—	++	++	±	—	++	++	+
Walker	++	++	++	—	++	++	++	—	++	++	++	—
Thorpe	++	++	++	—	++	+	++	—	++	+	++	—
Control strains												
Type 10	++	±	—	—	++	Ag.	—	—	++	SLAg.	—	—
" 20	++	++	—	—	Ag.	++	++	—	SLAg.	++	—	—
" 29	—	—	++	—	—	—	++	—	—	—	++	—
" 31	—	—	—	++	—	—	—	+	—	—	—	++

++ = maximal capsular swelling.
 + = slightly submaximal swelling.
 ± = definite swelling.

— = doubtful swelling, moderate to heavy agglutination.

Ag. = moderate to heavy agglutination, no swelling.

SLAg. = slight agglutination, no swelling.

— = no agglutination, no swelling.

this group of cultures with the 3 sets of sera are close enough to strict specificity to furnish further evidence of the multiple-antigenicity of the test-strains. The somewhat discrepant results obtained with the sera of the 3 manufacturers may have either of two explanations: (1) cross-reacting antibodies may have been more completely removed from one set of sera (by absorption) than from another, or (2) more strictly specific cultures may have been used as antigens in the production of one set of sera than in another.

While measurement by loops, as in the Neufeld test, is not a perfectly quantitative method, the results here cited have been obtained so repeatedly and consistently that they leave no room for doubt as to their reproducibility.

From the figures cited above it seems likely that the occurrence of these broadly non-specific strains is fairly frequent. Their significance for the prevailing conception of type-specificity is obvious. Recognition of their existence creates a number of problems in the diagnosis and treatment of pneumonia, some of which are being studied further.

